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(71) Applicant: **AMERICAN CYANAMID COMPANY**
One Cyanamid Plaza
Wayne New Jersey 07470 (US)

(72) Inventor: **Kreitman, Martin**
5760 S. Blackstone Avenue
Chicago, Illinois 60637 (US)
Inventor: **Taylor, Martin**
2246 East 6th Street
Tuscon, Arizona 85719 (US)
Inventor: **Black, Bruce C.**
286 Forest Road
Yardley, Pennsylvania (US)

(74) Representative: **Wächtershäuser, Günter, Prof. Dr.**
Patentanwalt
Tal 29
D-80331 München (DE)

(54) Method for monitoring pesticide resistance.

(57) The present invention relates to an isolated nucleic acid fragment comprising a nucleic acid sequence encoding a lepidopteran sodium channel, or portion thereof.

EP 0 615 976 A1

Each year, approximately one third of the world's crops are destroyed by plant pests, amounting to billions of dollars in crop losses in the united States alone. Plants are susceptible to diseases and damage caused by an enormous number of different types of organisms, including virus, bacteria, fungi, algae, parasitic plants, weeds, insects, arachnids, and nematodes. The potential losses are kept in check by natural controlling mechanisms, and when these systems fail, by applications of various types of insecticides which typically act by attaching one specific, genetically controlled aspect of the target organism's metabolism. However, the efficacy of any given pesticide may be limited by the appearance and spread of resistance to the pesticide among the target population. The appearance and spread of insecticide resistance in wild populations argues for a genetic origin. First, a resistant genotype or trait appears in a local population and then with continued insecticide use (and thus, disproportionate survival of individuals with this genotype or trait), the resistance rapidly increases in the population and via migration resistance may spread to regional and perhaps even worldwide populations. Resistance may arise as a genetic allele already present within a population, or it may arise de novo. Nonetheless, whatever the cause, in a population with a short generation time (which is characteristic of many insects), the resistance trait can spread rapidly and quickly render ineffective the planned pattern of pesticide application.

The continued development of natural strategies for insect control could be enhanced by an understanding of the genetic basis of the resistance in economically important pests. Such studies have been ongoing, particularly with regard to insect pests, and a great deal has been learned about the major types of resistance observed in insects. At least three types of insect resistance have been identified: decreased rate of uptake, increased rate or degradation and changes in the target site. To some extent, certain aspects of the genetic mechanisms of these types of resistance have been determined; however, knowledge of the specific genetic basis for resistance has not yet been effectively applied in the field to monitor the occurrence of resistance, or to assist in planning effective insecticide applications to avoid or alleviate the development of resistance. Modification of insecticide application patterns can be critical in cases in which resistant insects are otherwise less fit than non-resistant insects; application of insecticide to which some individuals are resistant in these cases may actually select for increase in resistance in the population, when it might otherwise have been maintained only at low levels or entirely eliminated from the population. Thus, a method for exploiting the available knowledge of the genetic basis for resistance is greatly needed.

Some of the most destructive of insect pests are found among the order Lepidoptera. The damage caused by lepidopterans is most frequently related to feeding activity of their larvae (caterpillars) on plants. Of the lepidopteran plant pests, among the most damaging are those insects related to the genus Heliothis. Two species of the genus Heliothis, H. virescens (the tobacco budworm) and H. armigera (American bollworm), and Helicoverpa zea (the corn ear worm) are responsible for a tremendous amount of damage to tobacco, cotton, corn, beans, alfalfa, and solanaceous plants in the United States. Over the years these pests have been controlled by application of a variety of insecticides; however, H. virescens has regularly developed resistance to compounds from virtually every major insecticide class. As one exception, until fairly recently the pyrethyroid class of insecticides continued to effectively control Heliothis in the field. Unfortunately, it has recently been noted that pockets of tolerance or resistance are beginning to appear in Heliothis virescens populations in various areas in the United States and in H. armigera and H. punctigera abroad. Because pyrethroids represent the most effective control of these insects, it is essential that widespread occurrence and/or spread of resistance to pyrethroids be avoided.

Resistance to pyrethroids has been extensively studied in a variety of dipterans, and a number of different patterns of inheritance and explanations for resistance have been suggested. However, the basis for pyrethroid resistance or tolerance in lepidopterans generally, and in Heliothis specifically, has not yet been clarified. An understanding of the genetic mechanism of resistance, or even a definable genetic marker for resistance, would provide a much-needed basis for tracking the resistance trait accurately in a population. The present invention now provides the necessary tools for monitoring the occurrence and spread of resistance in a population, in particular for pyrethroid resistance in lepidopteran populations.

SUMMARY OF THE INVENTION

The present invention provides an isolated nucleic acid fragment encoding all or a portion of a non-dipteran sodium channel. This channel is believed to be target site for sensitivity to a variety of different insecticides, including pyrethroids, and is useful as a marker for such target-insensitive insecticide resistance. Preferably the fragment encodes a lepidopteran, coleopteran or homopteran sodium channel. Sodium channels from both resistant and sensitive strains are encompassed herein. The nucleic acid fragment provides the basis for probes useful in detecting the presence of the resistance trait in a population of insects to be evaluated. Also provided are vectors containing the resistance gene which may

be used to introduce a gene encoding insecticide resistance into beneficial insects, such as honey bees. The invention also provides the isolated protein or fragment encoded thereby, as well as biologically or immunologically active fragments thereof, which protein or fragments are useful in generation of polyclonal and monoclonal antibodies. Such antibodies can be used to detect the presence of sensitive or insensitive sodium channels. In a preferred embodiment, the insecticide target is a Heliothis sodium channel.

The invention also provides a means for monitoring, both quantitatively and qualitatively, the level of resistance in any given pesticide target population. The presence or absence of a resistance trait is determined by hybridizing whole genomic DNA, cDNA or one or more restriction fragments from one or more individuals from the population with a nucleic acid probe based on the sequence of a nucleic acid encoding a pesticide target site. Quantification of the trait is further obtained by calculating the number of the individuals having resistance relative to the number of sensitive individuals, and calculating the percentage occurrence of resistance. This in turn permits the observer to determine whether or not the contemplated pesticide application will be effective, whether alternate treatment may be required, or to predict when, at some time in the future, alternate treatment may be needed. In an alternate embodiment, the DNA can be used to express a recombinant protein or peptide, which in turn can be used to raise monoclonal antisera. Preferably antisera that can specify or identify both resistant and sensitive targets are raised. Such monoclonal antibodies may then be utilized in routine immunological procedures to determine the presence or absence of the resistant protein in a population.

The present invention also provides the basis for an in vitro screen which will detect potential insecticidal activity. A nucleic acid sequence encoding a lepidopteran sodium channel can be inserted into a convenient host cell and a battery of potential insecticides tested for their ability to interfere with expression of either the gene or the encoded protein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the nucleotide and amino acid sequences of the Heliothis clone hscp1, in comparison with the nucleotide and amino acid sequence of the para locus (sodium channel) of Drosophila melanogaster. "Dm" = Drosophila sequence; "scd" = portions of the Heliothis sequence; the numbers after "scd" refer to various subclones used to determine the sequence. The underlined amino acid sequences are membrane-spanning domains of the sodium channel. Superimposed above the sequences are the specific sequences of various primers (e.g. HSC 3455+) used in cloning and/or sequencing procedures. Numbering is based on the Drosophila homologue sequence to the Heliothis sodium channel.

Figure 2 shows Restriction Fragment Length Polymorphisms (RFLPs) developed utilizing a labelled hcsp1 DNA sequence as a probe. "RR" identifies DNA derived from resistant individuals and "SS" refers to DNA derived from sensitive individuals. The presence or absence or resistant and sensitive individuals is made by the vial test described by Campanhola and Plapp, J. Econ. Entomol., 82:1577-1533, 1989. Protocols for the procedure are described in Example 3.

DETAILED DESCRIPTION OF THE INVENTION

As described in detail in the following Examples, the Heliothis sodium channel is isolated by amplification of Heliothis genomic DNA from an inbred susceptible strain using degenerate primers homologous to a portion of a sodium channel gene from Drosophila melanogaster (Loughney et al. Cell 58:1143-1154, 1989), as described in Example 2. A 184 bp amplification product is obtained which, upon sequencing, is found to encode an identical amino acid sequence when compared to the same region in the Drosophila gene. This PCR product is then labelled and hybridized to restriction enzyme-digested Heliothis genomic DNA. The highest molecular weight DNA fragment identified is from an EcoRI digest.

Genomic DNA is then isolated from a resistant Heliothis strain and digested to completion with EcoRI. A genomic library is constructed in a g Zap II vector, and a labelled 184 bp fragment is then used to screen this library. One positive plaque yields a genomic clone of approximately 8000 bp which is referred to as "hscp1." This clone shows significant homology to the published Drosophila sequence (Figure 1).

Based on the hspc 1 sequence, a pair of primers designated 4116+, and 4399- (as depicted in Figure 1) are used to amplify fragments of the sodium channel gene from both resistant and susceptible Heliothis individuals. Fragments are digested with either RsaI, Sau3AI or MseI. The restriction fragments are then separated and analyzed by gel electrophoresis. The resulting Restriction Fragment Length Polymorphisms (RFLPs) show distinct patterns unique to resistant and susceptible individuals. This demonstrates the utility of a nucleic acid sequence for defining genetic RFLP patterns useful for identifying resistant individuals within a population (Figure 2).

By homology with the known nucleic acid sequence for a Drosophila sodium channel, it is presumed that the isolated Heliothis sequence represents a portion of the corresponding Heliothis channel. Also, by comparison with the available information regarding the Drosophila channel as being the target site of pyrethroid action, it is reasonable to extrapolate this function in Heliothis as well. However, whether or not the isolated sequence represents the target site, or a genetic locus that is tightly linked with resistance, the RFLP results described above show that difference in the DNA is a reliable marker for identifying differences in susceptibility to insecticides that primarily target the sodium channel, particularly pyrethroids (but also chlorinated hydrocarbons and venom components such as the toxin derived from Androctonus australis [Aalt], saxitoxin, tetrodotoxin and the like) in an insect population.

The isolation of the DNA sequence encoding the Heliothis sodium channel provides a number of advantages. First, in view of the unexpected high level of homology between Drosophila and Heliothis sodium channels, it must be assumed that channels of other lepidopteran species have similar or even higher homology to the Heliothis sodium channel. Thus, the Heliothis sodium channel DNA provides the basis for isolation of other lepidopteran channels. Such lepidopteran channels can be readily isolated by hybridization under medium (e.g., 1xSSC, 0.1% SDS, 55°C) or high (0.1 x SSC, 0.1% SDS, 65°C) stringency conditions using the Heliothis DNA or portion thereof, to function as an identifiable probe when screened against cDNA or whole genomic libraries from the species of interest. Isolation of DNA hybridizing under said conditions can be achieved by standard techniques. Lepidopteran species of interest include, but are not limited to: other Heliothis species, such as the American bollworm, H. armigera and the bollworm, H. punctigera; lepidopteran species of the genus Spodoptera, e.g., the Egyptian cotton leafworm, S. litteralis, the beet armyworm, S. exigua; the fall armyworm, S. frugiperda; the cutworm, S. litura, the rice swarming caterpillar, S. mauritania and the Southerm armyworm, S. eridania; and other miscellaneous lepidopterans, e.g., the pink bollworm, Pectinophora gossypiella; the spiny bollworm, Earius insulana, the cotton leafworm, Alabama argillacea; the leaf perforator, Bucculatrix thurberiella; the tomato fruitworm, Helicoverpa zea; the diamondback moth, Plutella xylostella; the cabbage looper, Trichoplasia ni; the imported cabbageworm, Artogeia rapae; the imported cabbageworms Hellula undalis and Hellula rogatalis; the black cutworm, Agrotis ipsilon; the corn earworm, Ostrinia nubalis; the tomato pinworm, Keiferea lycopersicella; the tomato hornworm, Manduca sexta and Manduca guinquemaculata; the velvet bean caterpillar, Anticarsia gemmatalis; the green oliveworm, Plathypena scabra; the soybean looper, Pseudoplusia includens; the saltmarsh caterpillar, Estigmene acrea; the leaf miner, Epinotia meritana; the codling moth, Cydia pomonella; the oblique banded leafroller, Choristoneura rosaceana; grape berry moth, Lobesia botrans; currant tortrix, Pandemis cerasana; spotted tentiform leafminer, Phyllonocytes blancardella; grape leafroller Sparganothis pilleriana; tufted bud apple moth, Platynota idacusalis; red banded leafroller, Argyrotaenea velutinana; oriental fruit moth, Grapholitha molesta; Southwestern corn borer, Diatraea grandiosella; rice leafrollers, Cnaphalocrocis medinalis, Marasmia exigua and Marasmia patnalis; striped borer, Chilo suppressalis; dark headed stem-borer, Chilo polychrysis; yellow stem borer, Scirophaga incatulas; white stem borer, Scirophaga innotata; and pink stem borer, Sesamia inferens.

The isolated Heliothis nucleic acid fragment is also useful in other regards. The newly observed homology between Drosophila and Heliothis sodium channels predicts not only substantial homologies between Heliothis channels and other lepidopteran species, but also between Heliothis and other non-lepidopteran insect channels. Thus, the fragment, or portions thereof, can be utilized in developing RFLP's for other lepidopteran species, including, but not limited to, e.g., the lepidopteran species noted above, as well as non-lepidopteran species such as as the Colorado potato beetle Leptinotarsa decimlineator, the boll weevil, Anthonomus grandis; the Southern corn rootworm, Diabrotica undecimpunctata; the Japanese beetle, Popillia japonica; plum curculio, Conotrachelus nenuphar; brown planthopper, Nilaparvata lugens; green leafhopper, Nephotettix virescens; potato leafhopper, Empoasca abrupta; cotton aphid, Aphis gossypii; green peach aphid, Myzus persicae; sweetpotato whitefly, Bemisia tabaci; imported fireant, Solenopsis invicta; thrips, e.g., Thrips palini; pear psylla, Psylla pyri; two-spotted spider mite, Tetranychus urticae; carmine mite, Tetranychus cinnabarinus; citrus rust mite, Phyllocoptruta oleivora; German cockroach, Blatella germanica; cat flea, Ctenocephatides felis; yellow fever mosquito, Aedes aegypti; and salt marsh mosquito, Aedes sollicitans. The generation of useful RFLPs for these species is achieved in substantially the same manner as described herein for Heliothis.

The Heliothis nucleic acid fragment or portions thereof can also be used as a probe, or can be used as the basis for designing degenerate probes, to screen genomic or cDNA libraries derived from such other non-lepidopteran insect species for specific sodium channels from these species. However, given the herein demonstrated high level of homology between the distantly related Drosophila and Heliothis, it is quite likely that the present Heliothis virescens fragment can be used directly as a probe for identifying resistant sodium channels by RFLPs for other lepidopteran and nonlepidopteran species, without the need for

isolation of those species' specific sodium channel DNA fragments.

Continued monitoring and early detection of the presence of a resistance trait in any population is essential to effective insect control. By the time resistance is apparent at the gross level, it is very likely already at a point where further treatment with the pesticide is doomed to failure. For example, application of pyrethroids to a population in which resistance is already established will substantially increase the selection pressure favoring the appearance of the resistance trait. Whereas, in the absence of such selection, the resistant individuals are reproductively less fit than sensitive (wild-type) individuals. Hence, resistance would not otherwise have become established in the population without the application of insecticides. Thus, selective and timely application of pesticides or recognition of need for alternative application of pesticides at an early stage can be critical in maintaining suitably sensitive insect populations.

The identification of a genetic trait associated with resistance provides several avenues for tests to monitor the occurrence and frequency of resistance in a population at a very early stage, when frequency may be low and/or undetectable by standard bioassays. Early observance permits for informed judgments in the application of the relevant pesticide. For example, the gene encoding the resistant sodium channel provides the basis for informative southern or RFLP analysis of an insect population to identify the presence of the resistance trait in a given population. Detection of the unique DNA associated with a resistance allele (or the presence of two distinct alleles) therefore is diagnostic for the presence of the resistance trait in an analyzed sample. This may be determined, for example, by digesting genomic DNA collected from individuals of the target population in question and probing a Southern blot with detectably labelled DNA sequence that identifies a particular resistance trait, or a diagnostic portion thereof, to identify the presence or absence of the resistance allele. By "diagnostic portion" thereof is meant any fragment of the hscp1 DNA which differs sufficiently in sequence from the corresponding portion of the susceptible DNA sequence, or a unique DNA sequence genetically linked to the trait, so as to assure its hybridization, under high stringency conditions, only with DNA encoding the resistance trait. It should be noted that sequences flanking the resistance gene, as well as intervening sequences (introns) are particularly suited for identifying unique diagnostic RFLPs.

RFLP analysis also provides an attractive method of analyzing the existence and frequency of the resistance trait in the population. As the Examples below show, there is a detectable polymorphism associated with the sodium channel DNA between resistant and susceptible individuals. Thus, target population DNA can be analyzed for the presence of polymorphisms using the detectably labelled cloned hscp1 DNA as a probe. In this technique, DNA from several individuals in the target population is digested with an appropriate restriction enzyme, and size separated by gel electrophoresis. The gel, or a blot derived therefrom, is then probed with labelled DNA, either the whole gene or fragment. If there are both resistant and sensitive alleles within an individual in the population, there will appear on the gel two different sized restriction fragments, each of which will hybridize with the hscp1 probe. In this manner, large numbers of individuals in the population can be sampled, and the relative abundance of the allele can be determined. Identification of the specific DNA fragment associated with resistance, whether by Southern or RFLP analysis, will always be diagnostic.

In this regard, the present invention also provides a kit for evaluating the extent to which a resistance gene is present in a given population. The kit will contain as its principle components (1) a restriction enzyme for digesting DNA, and (2) a detectably labelled probe comprising a nucleic acid fragment capable of hybridizing specifically with DNA encoding the resistance trait, or a nucleic acid fragment capable of hybridizing with the diagnostic RFLP marker. In a preferred embodiment, the kit also comprises (3) a means for extracting DNA from cells of the target population, and/or (4) PCR primers useful in amplifying the target DNA sequences. Also included may be a set of reference standards comprising sensitive and resistant DNA.

As a specific example, a kit for the detection of altered sodium channels in a population would include (1) a restriction enzyme such as TagI or EcoRI, which will generate fragments which show the relevant polymorphism, if present (2) a radioisotope-or biotin- labelled DNA comprising the sequence of the sodium channel or fragments thereof; and optionally (3) a DNA extraction means.

It will be recognized by those skilled in the art that variations or components (1) and (2) in particular are contemplated. Any restriction enzyme which produces a detectable polymorphism can be used. Preferably, the enzyme used will be a 4-cutter, such as Sau96I, ScrFI, Sau3A1, RsaI, MseI, MspI, MboI, HpaII, HinPI, HaeIII, DpnII, BstVI, and BfaI; or a 6-cutter, such as EcoRI, BamHI, HindIII, PstI, and SalI; less useful are 8-cutters, such as NotI, StoI, PacI, Sse36I, AscI, FseI, PmeI, RsrII, or SwaI. The utility of any given restriction enzyme can readily be determined by digesting DNA known to contain alleles for both resistance and sensitivity with the candidate enzyme, and observing the presence or absence of a polymorphism by probing with hscp1, or any DNA linked to this region. Also, it will be understood that the "detectably

labelled" DNA may alternately be labelled so as to be detectable in any manner known in the art, e.g., by chemiluminescence, bioluminescencse, ELISA, biotinavidin, or any other appropriate means. The foregoing scheme is useful for detecting the presence of resistance to not only pyrethroids, but also DDT and arthropod toxins, such as the sodium channel toxin derived from Androctonus australis (AaIT).

Those skilled in the art will also recognize that the approach to resistant pest management described herein is not limited solely to control of resistance based on an altered sodium channel. Utilizing target site DNA as a means of tracking the presence of resistance in a population provides a far more precise and sensitive measure of the prevalence of resistance than do previously utilized methods. The target sites for many types of pesticides are now known, and therefore, the proposed genetic analysis for a resistance trait can be applied to other insecticides as well. For example, acetylcholinesterase is known to be the target site for carbamate and organophosphate insecticides (Oakeshott et al., PNAS USA 84:3359-3363, 1987). Organophosphate insecticides include malathion, methylparathion, diazinon, turbophos and dicrotophos; carbamates include sevin, Aldicarb, methionyl and thiodicarb. Target site resistance to some of these insecticides has been reported (Karunaratre et al., Resist. Pest. Manag. Newsletter, 3:11-13, 1991; Chen, Resist. Pest Manag. Newsletter, 2:15, 1990). The acetylcholinesterase gene has been cloned (Fournier et al., J. Mol. Biol. 210:15-22, 1989), providing the basis for development of an analogous detection system for this type of resistance . Monooxygenase and mixed function oxidases (MFOs) have also been shown to be involved in resistance by increase in the rate of metabolism of organophosphates and carbamates (Brattstein et al., Science, 1961349-1352, 1977; Brattstein et al., Pesticide Biochem. Physiol., 3:393, 1973, Krieger et al., science, 172:579, 1971; Matsumara, Toxicology Insecticides, Plenum Press, New York, 1975). Cyclodienes have been shown to act at the GABA receptor (Kadous et al., Pestic. Biochem. Physiol. 19:157-166, 1983; Tanaka et al., Pestic. Biochem. Physiol., 22:117-127, 1984); and target site resistance is known to exist (ffrench-Constant et al., J. Econ. Entomol. 83:1733- 1737, 1990) and the receptor gene has been cloned (french-Constant et al., PNAS USA, 88:7209-7213, 1991). Similarly, methoprene and certain botanical extracts (Precocenes) target the juvenile hormone (JH) receptor and resistance to these insecticides has been reported (Wilson et al., Devel. Biol., 118:190-201, 1986; Georghiou et al., J. Econ. Entomol., 71:544-547, 1978; Dyte, Nature, 238:48-49, 1972). Bacillus thuringiensis (Bt) toxins affect a gut associated glycoprotein but resistance has not become widespread. Diacyl hydrazine and certain botanical extracts (Penosterone A) target the ecdysone receptor (Wing, Science, 241:467-469, 1988; Spindler-Barth et al., Arch. Ins. Biochem. and Phys., 16:11-18, 1991; Cherbas et al., PNAS USA, 85:2096-2100, 1988) and the genes for the ecdysone receptor have also been cloned (Yao et al., Cell, 71:63-72, 1992; Koelle et al., Cell, 67:59-77, 1991).

The use of this method is also not limited to detection of insecticide resistance, but may be applied to any other pesticides, including herbicides, acaricides, fungicides, nematicides, and molluscicides. A number of genes conferring resistance to herbicides have been characterized. For example, altered acetohydroxy acid synthase genes are the basis of resistance to sulfonylureas and imidazolinone herbicides (EP Application No. 91 119 254.0; Yadav et al., PNAS USA 83:4418-4422, 1986). Glyphosate targets the enzyme 5-enolpyruvate shikimate-3-phosphoric acid synthase, and mutant genes encoding resistant forms of this enzymes have been identified (Comai et al., J. Biol. Chem., 260:4724-4728,1985). Similarly, genes conferring resistance to the herbicides phosphothrinicin and bialyphos have also been characterized (Thompson et al., EMBO J, 6:2519-2523, 1987; DasSarma et al., Science, 232:1242-1244, 1986).

The target site of various fungicides is also known. For example, phenylamide fungicides, such as acylalanines (metalaxyl, furaxyl and bevalaxyl), butrolactones (ofurase, cyprofuran), and oxazolidinones (oxadixyl) are known to act on fungal RNA polymerase (Arp et al., Fungizider. Mitt. Biol. Bundesanst 236-237, 1981; Davidse, Neth. J. Plant Pathol. 87:11-24, 1981; EPPO Bull 15:403-409, 1985). Resistance to these fungicides has also been reported (Davidse et al., J. Plant Pathol., 87:65-68, 1981; Davidse et al., Experiment. Mycology, 7:344-361, 1983). The fungicide carboxin is known to have as a target site succinate dehydrogenase (Schewe et al., in Modern Selective Fungicides, H. Lyr, ed. V.E.B. Gustan Fischer Vertag. Jene, 1987). Resistance and cloning of the resistance gene have also been reported (Keon et al., Current Genetics, 19:475-481, 1991). The blasticidin fungicides, such as BlaS and Blasticidin S act on the enzyme nucleoside aminohydrolase; resistance has been observed and the gene conferring the resistance has been cloned (Kamakura et al., Mol. Gen. Genet. 223:169-179, 1990; Kamakura et al., Agric. Biol. Chem., 51:3165-3168, 1987). The benzamidazole fungicides, such as benamyl, carbendazim, mocodazole and thiabenazole, act by affecting with microtubule function (Clemons et al., Pesticide Biochem. Physiol., 1:32-43, 1971; Hammersdag et al., Pesticide Biochem. Physiol., 3:42-54, 1973). Resistance is also known to occur to these fungicides (Van Tuyl, Med. Fac. Lonbouww Ryksuniv. Gent., 40:691-698, 1975); Meded. Landb. Hogesch. Wageningen, 77:1-137, 1977); Fanetran et al., Mycol. Res., 95:943-951, 1991). The relevant resistance gene has been isolated and cloned (Jang et al., Cell Motility and the Cytoskeleton, 17:87-94, 19906; Orbach et

al., Mol. Cell Biol., 6:2452-2461, 1986).

Other applications of this method will be apparent to those skilled in the art, in view of the following non-limiting examples.

EXAMPLES

1. DNA Preparation

Genomic DNA is prepared from adults of an inbred American Cyanamid Company susceptible strain of Heliothis virescens as follows. A moth is placed in 400 ml of grinding buffer (0.1 M Tris-HCl, pH 9.0, 0.1 M EDTA, 1% SDS) and homogenized with a pestle. 80 ml of 5M KOAc and 400 ml equilibrated phenol is added; the sample is inverted several times and left to stand on ice for five minutes. Two hundred ml of ice cold chloroform is added, spun at 15,000 x g for five minutes, and supernatant removed. The procedure is repeated at least once.

Four hundred ul chloroform is added to the pellet, the sample inverted for 30 seconds and then spun for 5 minutes at 15,000 x g. The chloroform is removed, the sample spun again for one minute and the remaining chloroform removed. Two volumes of cold ethanol are added to the aqueous phase, and the sample left to stand five minutes at room temperature. The sample is once again spun for five minutes, the supernatant aspirated, and the pellet dried. The dried pellet is resuspended in 50 ul Tris-EDTA (10mM TRIS, 1mM EDTA, pH 8.0).

2. Isolation of Channel Fragment from Genomic DNA

The isolated genomic DNA is used as a template in PCR with primers based on portions of the Drosphila melanogaster para locus sodium channel.

Specifically, degenerate primers homologous to portions of an exon in the fourth transmembrane domain of the a-subunit of the Drosophila para locus are constructed as follows:

para 4991+ 5′ (T3) GAAATCACTCCCAATTA ATH GAR AAR TAY TTY GT 3′

para 5143- 5′ (M13-40) TTTCCCAGTCACGAC ATN GCR AAD ATR AAC AT 3′

where H = A, C or T, R = A, G or T, Y=C or T, and N = any base. Numbers refer to 3' terminal base positions in the para sequence. Underlined sequences are universal primer tails T3 and M13 -40 respectively used for sequencing of product.

PCR reactions of 100 ul are constructed of approximately 1 mg of genomic DNA, 1 mg of each primer, 0.2 mM of each dNTP, 10 mM Tris pH 8.3, 50 mM KCl, 2mM $MgCl_2$, 0.001% gelatin, and 2 U of Taq polymerase. Reactions are incubated for 5 cycles, each of 50 seconds at 94°C, 2 minutes at an annealing temperature of 53°C, and 25 seconds at 72°C, then for 35 cycles with an annealing temperature of 45°C. An amplification product of 184 base pairs is obtained, and then directly sequenced using the Sequenase kit (United States Biochemical Co.) according to the manufacturers directions. The deduced amino acid sequence is found to be the same as for an equivalent region in para.

Genomic DNA is also digested with several restriction enzymes, specifically EcoRI, BamHI, SalI, HindIII, PstI, and XbaI. The fragments are separated on agarose gel and transferred to a nylon support. The PCR product described above is radiolabelled and hybridized to the nylon blot at 60°C overnight. The blot is washed with a wash buffer (IMNaPi, 250 mM EDTA, pH8, 20% SDS; Napi = $Na_2HP0 \cdot 7H_20$, 134g and H_3PO_4 to pH7.2/liter) at 60°C three times for thirty minutes each. The filter is exposed to film. The film is developed after 12-24 hours of exposure at -80°C. The results show single bands in each lane indicative of a single copy gene. The largest band is for the EcoRI digest.

Based on the foregoing information genomic DNA is prepared from an ICI America's pyrethroid resistant PEG-87 H. virescens strain using cesium chloride purification as described by Ausubel et al. (Current Protocols in Molecular Biology, Green Publ. Assn. and Wiley Interscience, 1989), and digested to completion with EcoRI. This DNA is used to construct a genomic library in the Lambda-ZapII vector (Stratagene Co., LaJolla, CA) following manufacturers' instructions. The 184 bp PCR fragment is used to screen this library by hybridization as described in standard Lambda-Zap II protocols. Several positive plaques are purified and the inserts excised in vitro following manufactuer's instructions, and subsequently

characterized. A genomic clone designated "hscp1" has approximately 8000 bp, and is extensively sequenced. For this first 990 base pairs of coding sequence, there is significant homology between hscp1 and the published para sequence of Drosophila (Loughney et al., Cell, 58:1143-1154, 1989).

3. RFLP Analysis

Fragments of the gene from individuals of both ICI- pyrethroid-resistant lines and American Cyanamid Company susceptible strains (collected Stoneville, Mississippi, 1963) are amplified by PCR using several pairs of primers based on the available hscp1 sequence. In this specific example, hscp4116+ and hscp4399- are used. The PCR reactions, of 100 ml, consist of 100 ng-1mg of genomic DNA, 100 ng each of primer (hscp 4116+, 4399-, as shown in Figure 1) and other components as described above. Negative and positive control reactions are also made respectively, without template DNA or with hscp1 DNA.

Reactions are incubated for 30 cycles, each of 50 seconds at 94°C, 2 minutes at an annealing temperature of 56°C, and 1.5 minutes at 72°C. PCR products are purified with phenol, chloroform and precipitated using ammonium acetate-ETOH. PCR products are then apportioned among three different restriction enzyme reactions mixes following manufacturers' instructions (RsaI, Sau3AI, and MseI, New England Biolabs, Beverly MA), and incubated at 37°C overnight. Digestion products are resolved on a 3% "NuSieve" (FMC) agarose gel and stained with ethidium bromide at about 50ng/ml. The resulting restriction fragments length polymorphisms show a distinct pattern for each of the resistant and susceptible strains (Fig. 2), indicating the utility of this method in detecting the presence of resistant individuals among a generally susceptible population.

DEPOSIT OF BIOLOGICAL MATERIALS

The following materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, on October 19, 1992 and have been given the following accession numbers.

Deposit	Accession No.
Sodium channel para homolog (3' half of gene) from Heliothis virescens ICI strain PEG-87 (hscp1)	ATCC 75334

FIGURE 1

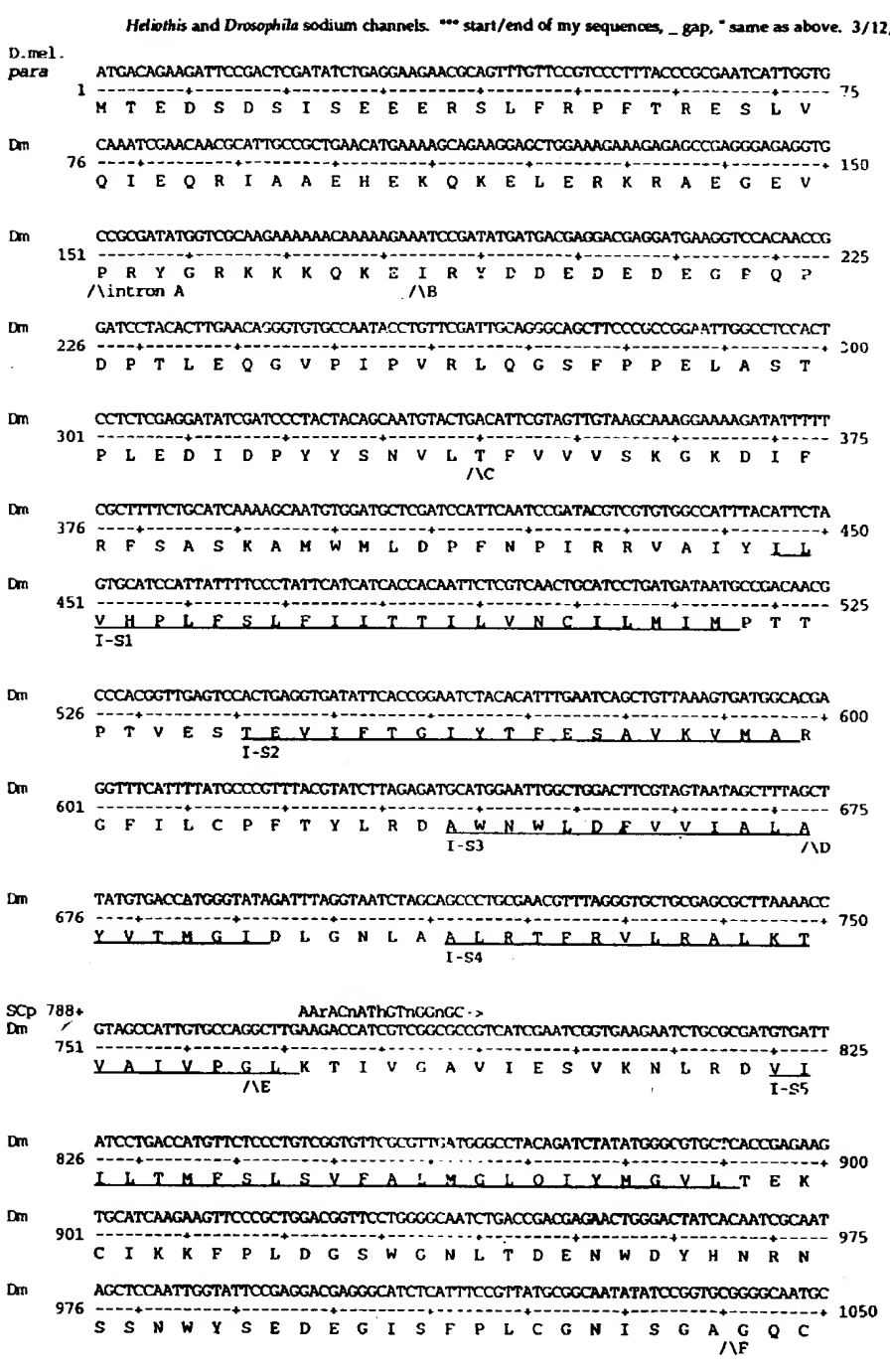

Heliothis and *Drosophila* sodium channels. *** start/end of my sequences, _ gap, ' same as above. 3/12/92 p1.
D.mel.
para
ATGACAGAAGATTCCGACTCGATATCTGAGGAAGAACGCAGTTTGTTCCGTCCCTTTACCCGCGAATCATTGGTG
M T E D S D S I S E E E R S L F R P F T R E S L V
Dm
CAAATCGAACAACGCATTGCCGCTGAACATGAAAAGCAGAAGGAGCTGGAAAGAAAGAGAGCCGAGGGAGAGGTG
Q I E Q R I A A E H E K Q K E L E R K R A E G E V
Dm
CCGCGATATGGTCGCAAGAAAAAACAAAAAGAAATCCGATATGATGACGAGGACGAGGATGAAGGTCCACAACCG
P R Y G R K K K Q K E I R Y D D E D E D E G F Q P
/\intron A
/\B
Dm
GATCCTACACTTGAACAGGGTGTGCCAATACCTGTTCGATTGCAGGGCAGCTTCCCGCCGGAATTGGCCTCCACT
D P T L E Q G V P I P V R L Q G S F P P E L A S T
Dm
CCTCTCGAGGATATCGATCCCTACTACAGCAATGTACTGACATTCGTAGTTGTAAGCAAAGGAAAAGATATTTTT
P L E D I D P Y Y S N V L T F V V V S K G K D I F
/\C
Dm
CGCTTTTCTGCATCAAAAGCAATGTGGATGCTCGATCCATTCAATCCGATACGTCGTGTGGCCATTTACATTCTA
R F S A S K A M W M L D P F N P I R R V A I Y I L
Dm
GTGCATCCATTATTTTCCCTATTCATCATCACCACAATTCTCGTCAACTGCATCCTGATGATAATGCCGACAACG
V H P L F S L F I I T T I L V N C I L M I M P T T
I-S1
Dm
CCCACGGTTGAGTCCACTGAGGTGATATTCACCGGAATCTACACATTTGAATCAGCTGTTAAAGTGATGGCACGA
P T V E S T E V I F T G I Y T F E S A V K V M A R
I-S2
Dm
GGTTTCATTTTATGCCCGTTTACGTATCTTAGAGATGCATGGAATTGGCTGGACTTCGTAGTAATAGCTTTAGCT
G F I L C P F T Y L R D A W N W L D F V V I A L A
I-S3
/\D
Dm
TATGTGACCATGGGTATAGATTTAGGTAATCTAGCAGCCCTGCGAACGTTTAGGGTGCTGCGAGCGCTTAAAACC
Y V T M G I D L G N L A A L R T F R V L R A L K T
I-S4
SCp 788+
Dm
AArACnATHGTnGGnGC ->
GTAGCCATTGTGCCAGGCTTGAAGACCATCGTCGGCGCCGTCATCGAATCGGTGAAGAATCTGCGCGATGTGATT
V A I V P G L K T I V G A V I E S V K N L R D V I
/\E
I-S5
Dm
ATCCTGACCATGTTCTCCCTGTCGGTGTTCGCGTTGATGGGCCTACAGATCTATATGGGCGTGCTCACCGAGAAG
I L T M F S L S V F A L M G L Q I Y M G V L T E K
Dm
TGCATCAAGAAGTTCCCGCTGGACGGTTCCTGGGGCAATCTGACCGACGAGAACTGGGACTATCACAATCGCAAT
C I K K F P L D G S W G N L T D E N W D Y H N R N
Dm
AGCTCCAATTGGTATTCCGAGGACGAGGGCATCTCATTTCCGTTATGCGGCAATATATCCGGTGCGGGGCAATGC
S S N W Y S E D E G I S F P L C G N I S G A G Q C
/\F

FIGURE 1

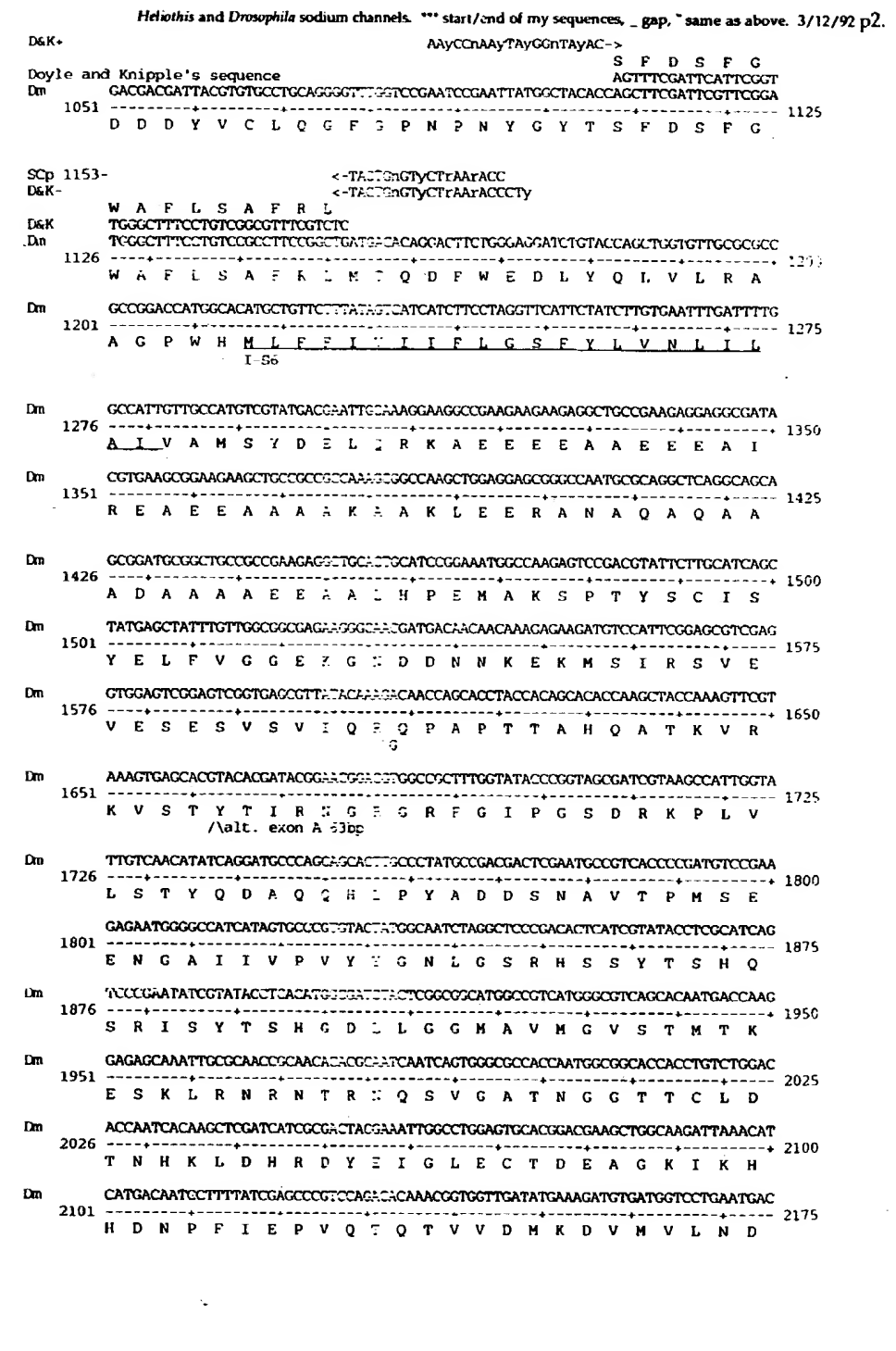

Heliothis and Drosophila sodium channels. *** start/end of my sequences, _ gap, " same as above. 3/12/92 p2.
D&K+
AAyCCnAAyTAyGGnTAyAC->
S F D S F G
Doyle and Knipple's sequence
AGTTTCGATTCATTCGGT
Dm
GACGACGATTACGTGTGCCTGCAGGGGTTTGGTCCGAATCCGAATTATGGCTACACCAGCTTCGATTCGTTCGGA
1051
1125
D D D Y V C L Q G F G P N P N Y G Y T S F D S F G
SCp 1153-
<-TACTGnGTyCTrAArACC
D&K-
<-TACTGnGTyCTrAArACCCTy
W A F L S A F R L
D&K
TGGGCTTTCCTGTCGGCGTTTCGTCTC
Dm
TCGGCTTTCCTGTCCGCCTTCCGGCTGATGTCACAGCACTTCTGGGAGGATCTGTACCAGCTGGTGTTGCGCGCC
1126
1200
W A F L S A F R L M T Q D F W E D L Y Q L V L R A
Dm
GCCGGACCATGGCACATGCTGTTCTTTATAGTCATCATCTTCCTAGGTTCATTCTATCTTGTGAATTTGATTTTG
1201
1275
A G P W H M L F F I M I I F L G S F Y L V N L I L
I-S6
Dm
GCCATTGTTGCCATGTCGTATGACGAATTGCAAAGGAAGGCCGAAGAAGAAGAGGCTGCCGAAGAGGAGGCGATA
1276
1350
A I V A M S Y D E L R K A E E E E A A E E E A I
Dm
CGTGAAGCGGAAGAAGCTGCCGCCGCCAAAGCGGCCAAGCTGGAGGAGCGGGCCAATGCGCAGGCTCAGGCAGCA
1351
1425
R E A E E A A A A K A A K L E E R A N A Q A Q A A
Dm
GCGGATGCGGCTGCCGCCGAAGAGGCTGCATCGCATCCGGAAATGGCCAAGAGTCCGACGTATTCTTGCATCAGC
1426
1500
A D A A A A E E A A L H P E M A K S P T Y S C I S
Dm
TATGAGCTATTTGTTGGCGGCGAGAAGGGCAACGATGACAACAACAAAGAGAAGATGTCCATTCGGAGCGTCGAG
1501
1575
Y E L F V G G E K G N D D N N K E K M S I R S V E
Dm
GTGGAGTCGGAGTCGGTGAGCGTTTACACAAGACAACCAGCACCTACCACAGCACACCAAGCTACCAAAGTTCGT
1576
1650
V E S E S V S V Y Q R Q P A P T T A H Q A T K V R
G
Dm
AAAGTGAGCACGTACACGATACGGAACGGACGTGGCCGCTTTGGTATACCCGGTAGCGATCGTAAGCCATTGGTA
1651
1725
K V S T Y T I R N G R R G R F G I P G S D R K P L V
/\alt. exon A 63bp
Dm
TTGTCAACATATCAGGATGCCCAGCAGCACTTGCCCTATGCCGACGACTCGAATGCCGTCACCCCGATGTCCGAA
1726
1800
L S T Y Q D A Q Q H L P Y A D D S N A V T P M S E
GAGAATGGGGCCATCATAGTGCCCGTGTACTATGGCAATCTAGGCTCCCGACACTCATCGTATACCTCGCATCAG
1801
1875
E N G A I I V P V Y Y G N L G S R H S S Y T S H Q
Dm
TCCCGAATATCGTATACCTCACATGGGATCTACTCGGCGGCATGGCCGTCATGGGCGTCAGCACAATGACCAAG
1876
1950
S R I S Y T S H G D L L G G M A V M G V S T M T K
Dm
GAGAGCAAATTGCGCAACCGCAACACACGCAATCAATCAGTGGGCGCCACCAATGGCGGCACCACCTGTCTGGAC
1951
2025
E S K L R N R N T R N Q S V G A T N G G T T C L D
Dm
ACCAATCACAAGCTCGATCATCGCGACTACGAAATTGGCCTGGAGTGCACGGACGAAGCTGGCAAGATTAAACAT
2026
2100
T N H K L D H R D Y E I G L E C T D E A G K I K H
Dm
CATGACAATCCTTTTATCGAGCCCGTCCAGACACAAACGGTGGTTGATATGAAAGATGTGATGGTCCTGAATGAC
2101
2175
H D N P F I E P V Q T Q T V V D M K D V M V L N D

Figure 1

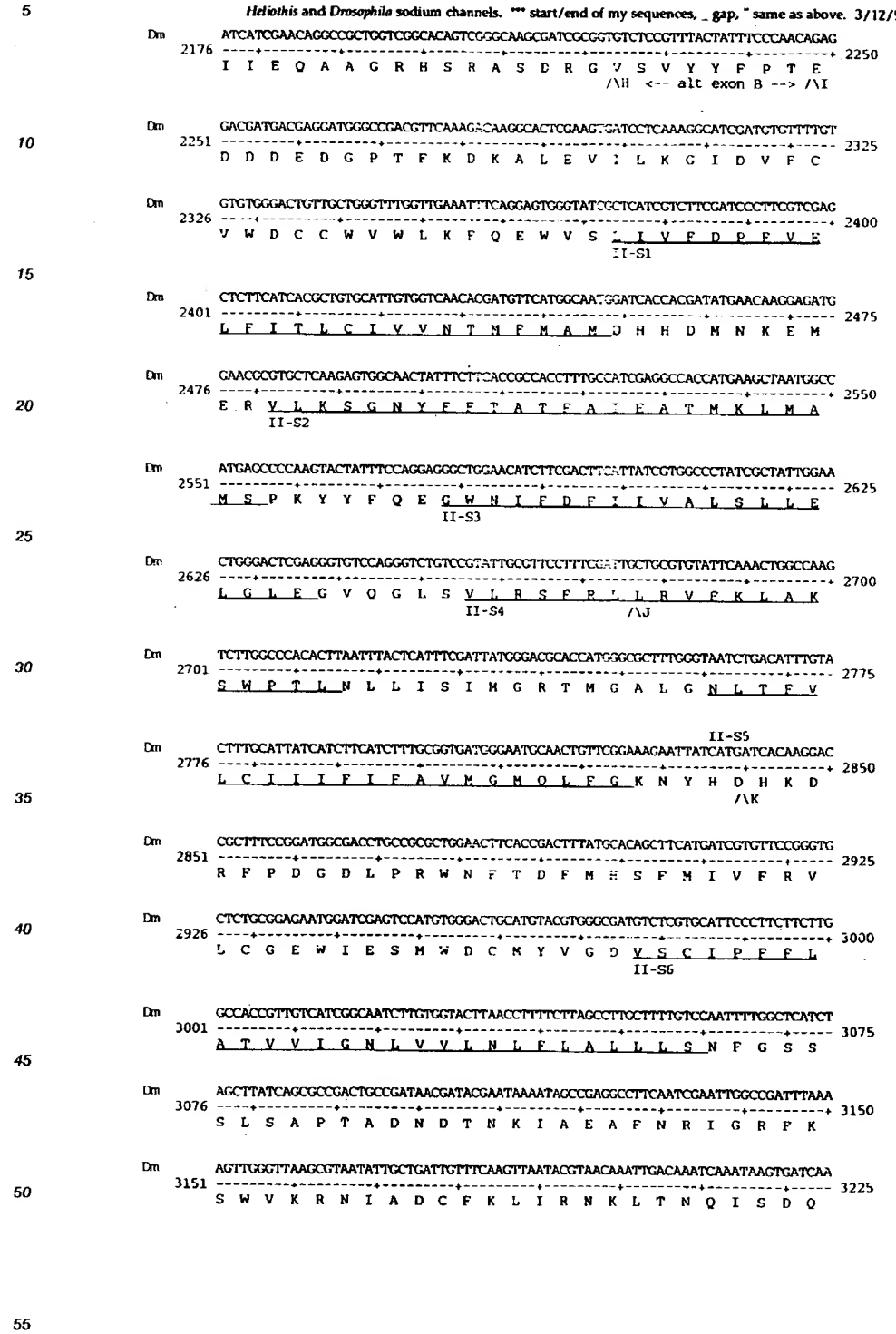

Heliothis and Drosophila sodium channels. *** start/end of my sequences, _ gap, " same as above. 3/12/92 p3.
Dm
2176
2250
I I E Q A A G R H S R A S D R G V S V Y Y F P T E
/\H <-- alt exon B --> /\I
2251
2325
D D D E D G P T F K D K A L E V I L K G I D V F C
2326
2400
V W D C C W V W L K F Q E W V S I I V F D P F V E
II-S1
2401
2475
L F I T L C I V V N T M F M A M D H H D M N K E M
2476
2550
E R V L K S G N Y F F T A T F A I E A T M K L M A
II-S2
2551
2625
M S P K Y Y F Q E G W N I F D F I I V A L S L L E
II-S3
2626
2700
L G L E G V Q G L S V L R S F R L L R V F K L A K
II-S4
/\J
2701
2775
S W P T L N L L I S I M G R T M G A L G N L T F V
II-S5
2776
2850
L C I I I F I F A V M G M Q L F G K N Y H D H K D
/\K
2851
2925
R F P D G D L P R W N F T D F M H S F M I V F R V
2926
3000
L C G E W I E S M W D C M Y V G D V S C I P F F L
II-S6
3001
3075
A T V V I G N L V V L N L F L A L L L S N F G S S
3076
3150
S L S A P T A D N D T N K I A E A F N R I G R F K
3151
3225
S W V K R N I A D C F K L I R N K L T N Q I S D Q

FIGURE 1

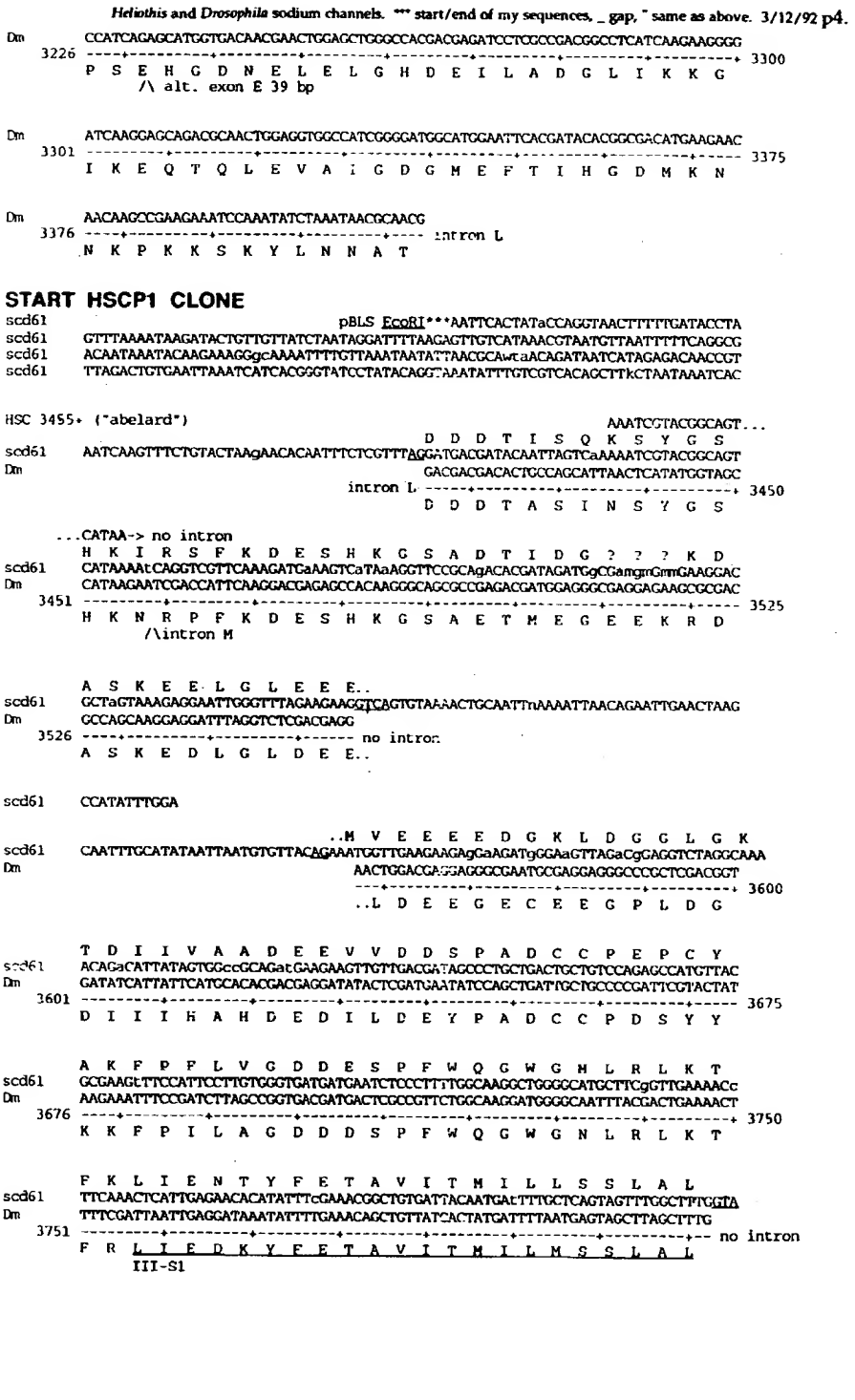

Heliothis and Drosophila sodium channels. *** start/end of my sequences, _ gap, " same as above. 3/12/92 p4.
START HSCP1 CLONE
pBLS EcoRI***AATTCACTATaCCAGGTAACTTTTTGATACCTA
HSC 3455+ ("abelard")
AAATCGTACGGCAGT...
intron L
...CATAA-> no intron
/\intron M
no intron
III-S1

FIGURE 1

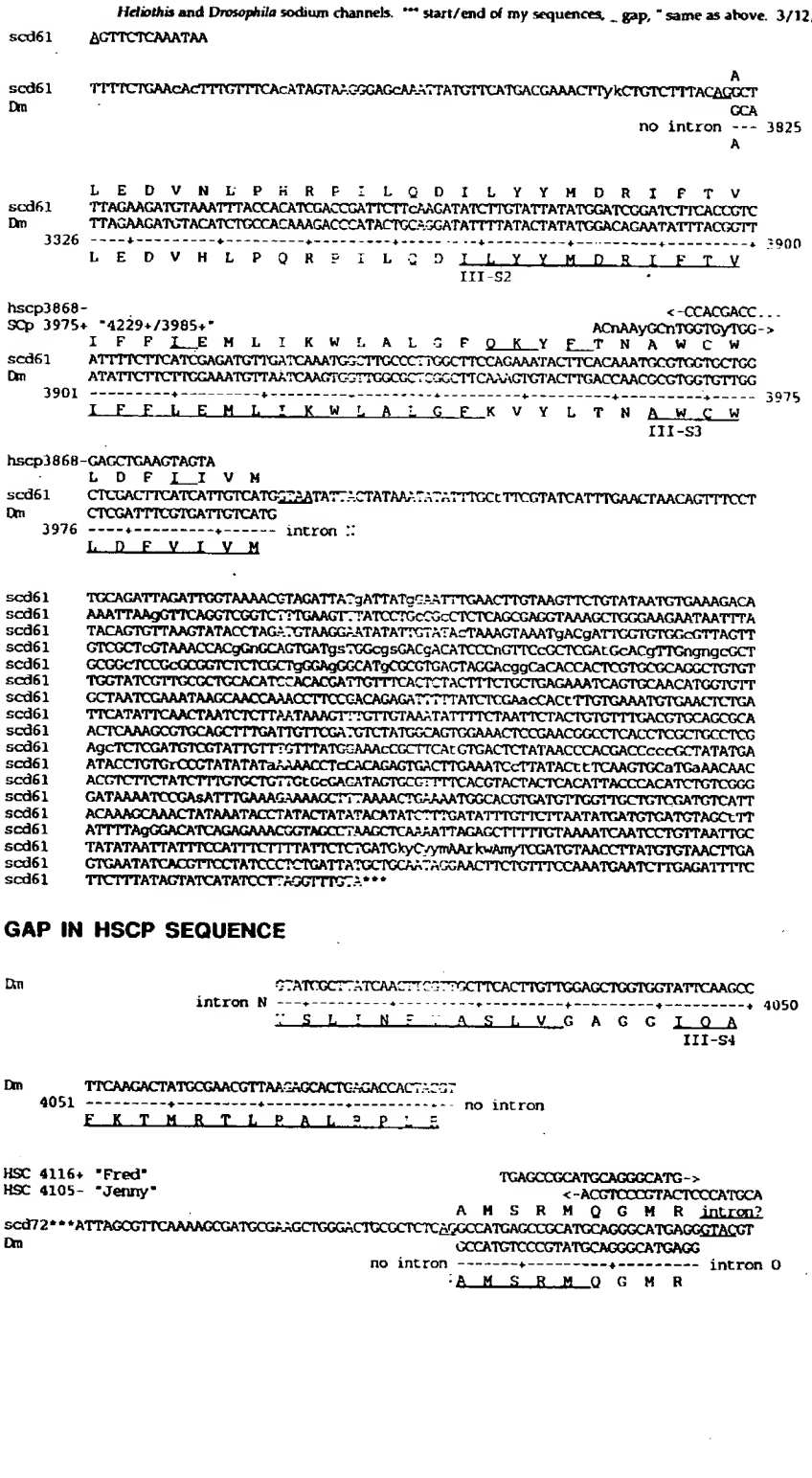

Heliothis and Drosophila sodium channels. *** start/end of my sequences. _ gap, " same as above. 3/12/92 p5.
scd61 ΔGTTCTCAAATAA
scd61 TTTTCTGAAcAcTTTGTTTCAcATAGTAAGGGAGcAAAGTATGTTCATGACGAAACTTykCTGTCTTTACAGGCT
Dm GCA
no intron --- 3825
A
L E D V N L P H R P I L Q D I L Y Y M D R I F T V
scd61 TTAGAAGATGTAAATTTACCACATCGACCGATTCTTcAAGATATCTTGTATTATATGGATCGGATCTTCACCGTC
Dm TTAGAAGATGTACATCTGCCACAAAGACCCATACTGCAGGATATTTTATACTATATGGACAGAATATTTACGGTT
3326 ----+---------+---------+---------+---------+---------+---------+---------+ 3900
L E D V H L P Q R P I L Q D I L Y Y M D R I F T V
III-S2
hscp3868-
SCp 3975+ "4229+/3985+" <-CCACGACC...
ACnAAyGCnTGGTGyTGG->
I F F I E M L I K W L A L G F Q K Y F T N A W C W
scd61 ATTTTCTTCATCGAGATGTTGATCAAATGGCTTGCCCTTGGCTTCCAGAAATACTTCACAAATGCGTGGTGCTGG
Dm ATATTCTTCTTGGAAATGTTAATCAAGTGGTTGGCGCTCGGCTTCAAAGTGTACTTGACCAACGCGTGGTGTTGG
3901 ---------+---------+---------+---------+---------+---------+---------+----- 3975
I F F L E M L I K W L A L G F K V Y L T N A W C W
III-S3
hscp3868-GAGCTGAAGTAGTA
L D F I I V M
scd61 CTCGACTTCATCATTGTCATGGTAATATTACTATAAATATATTTGCtTTCGTATCATTTGAACTAACAGTTTCCT
Dm CTCGATTTCGTGATTGTCATG
3976 ----+---------+------ intron ::
L D F V I V M
scd61 TGCAGATTAGATTGGTAAAACGTAGATTACgATTATgGAATTTGAACTTGTAAGTTCTGTATAATGTGAAAGACA
scd61 AAATTAAgGTTCAGGTCGGTCTTTGAAGTCTATCCTGcCGcCTCTCAGCGAGGTAAAGCTGGGAAGAATAATTTA
scd61 TACAGTGTTAAGTATACCTAGATGTAAGGAATATATTGTATAcTAAAGTAAATgACgATTGGTGTGGcGTTAGTT
scd61 GTCGCTcGTAAACCACgGnGCAGTGATgsTGGcgsGACgACATCCCnGTTCcGCTCGAtGcACgTTGngngcGCT
scd61 GCGGcTCCGcGCGGTCTCTCGCTgGGAgGGCATgCGCGTGAGTAGGAcggCaCACCACTCGTGCGCAGGCTGTGT
scd61 TGGTATCGTTGCGCTGCACATCCACACGATTGTTTCACTCTACTTTCTGCTGAGAAATCAGTGCAACATGGTGTT
scd61 GCTAATCGAAATAAGCAACCAAACCTTCCGACAGAGATTTTTATCTCGAacCACtTTGTGAAATGTGAACTCTGA
scd61 TTCATATTCAACTAATCTCTTAATAAAGTTTGTTGTAAATATTTTCTAATTCTACTGTGTTTGACGTGCAGCGCA
scd61 ACTCAAAGCGTGCAGCTTTGATTGTTCGATGTCTATGGCAGTGGAAACTCCGAACGGCCTCACCTCGCTGCCTCG
scd61 AgcTCTCGATGTCGTATTGTTTGTTTATGGAAAcCGCTTCAtGTGACTCTATAACCCACGACCcccGCTATATGA
scd61 ATACCTGTGrCCGTATATATaAAAACCTcCACAGAGTGACTTGAAATCcTTATACttTCAAGTGCaTGaAACAAC
scd61 ACGTCTTCTATCTTTGTGCTGTTGtGcGcAGATAGTGCGTTTTCACGTACTACTCACATTACCCACATCTGTCGGG
scd61 GATAAAATCCGAsATTTGAAAGAAAAGCTTTAAAACTGAAAATGGCACGTGATGTTGGTTGCTGTCGATGTCATT
scd61 ACAAAGCAAACTATAAATACCTATACTATATACATATCTTTGATATTTGTTCTTAATATGATGTCATGTAGCcTT
scd61 ATTTTAgGGACATCAGAGAAACGGTAGCCTAAGCTCAAAATTAGAGCTTTTTGTAAAATCAATCCTGTTAATTGC
scd61 TATATAATTATTTCCATTTCTTTTATTCTCTGATGkyCyymAArkwAmyTCGATGTAACCTTATGTGTAACTTGA
scd61 GTGAATATCACGTTCCTATCCCTCTGATTATGCTGCAACAGGAACTTCTGTTTCCAAATGAATCTTGAGATTTTC
scd61 TTCTTTATAGTATCATATCCTTAGGTTTGTA***
GAP IN HSCP SEQUENCE
Dm GTATGCTTATCAACTTGGTTGCTTCACTTGTTGGAGCTGGTGGTATTCAAGCC
intron N ---+---------+---------+---------+---------+---------+ 4050
Y S L I N F I A S L V G A G G I Q A
III-S4
Dm TTCAAGACTATGCGAACGTTAAGAGCACTGAGACCACTACGT
4051 ---------+---------+---------+---------+---------- no intron
F K T M R T L P A L P P I E
HSC 4116+ "Fred" TGAGCCGCATGCAGGGCATG->
HSC 4105- "Jenny" <-ACGTCCCGTACTCCCATGCA
A M S R M Q G M R intron?
scd72***ATTAGCGTTCAAAAGCGATGCGAAGCTGGGACTGCGCTCTCAGGCCATGAGCCGCATGCAGGGCATGAGGGTACGT
Dm CCCATGTCCCGTATGCAGGGCATGAGG
no intron -------+---------+--------- intron O
A M S R M Q G M R

FIGURE 1

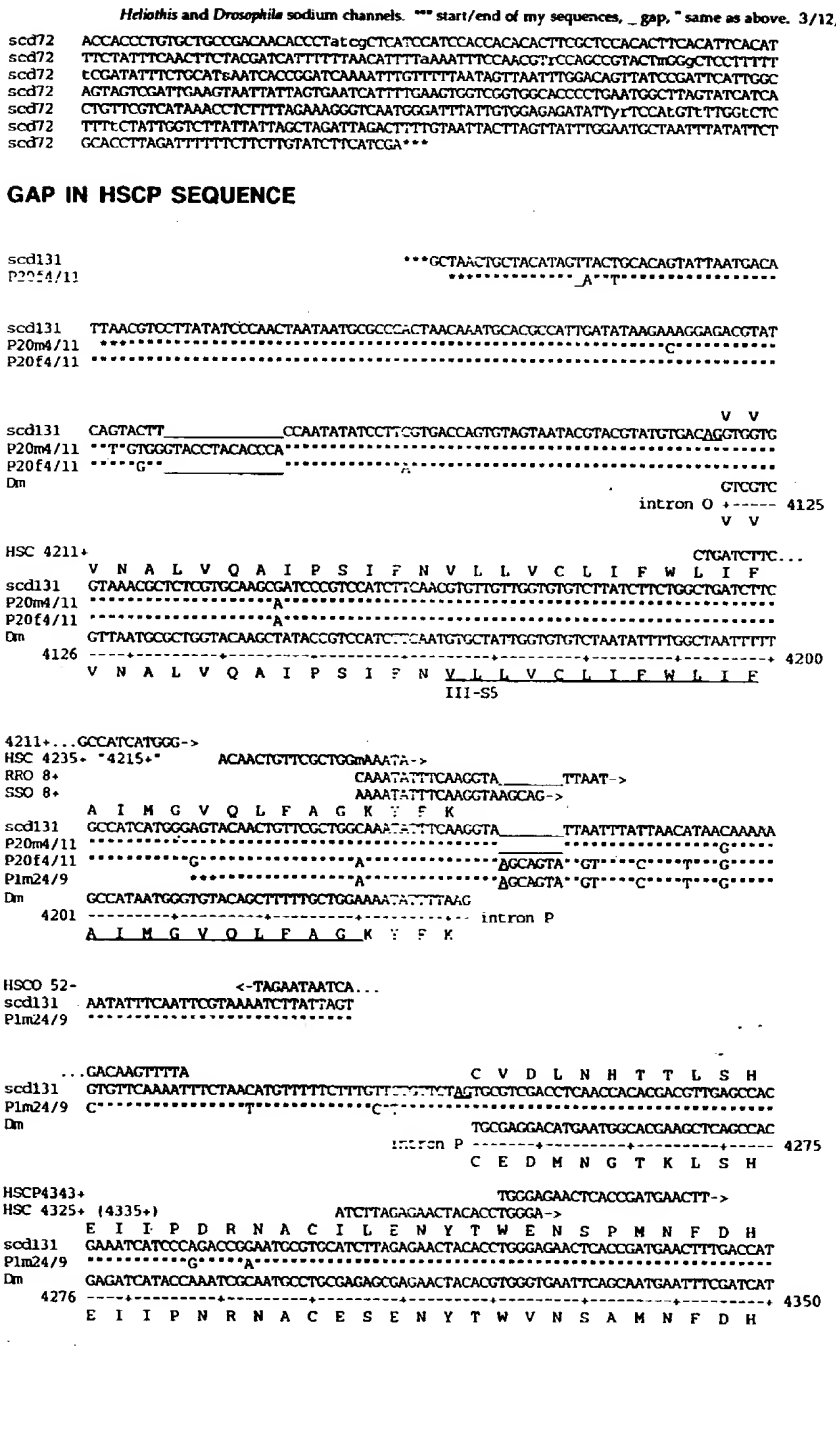

Heliothis and Drosophila sodium channels. "*" start/end of my sequences, _ gap, " same as above. 3/12/92 p6.
GAP IN HSCP SEQUENCE
intron O
intron P
III-S5

FIGURE 1

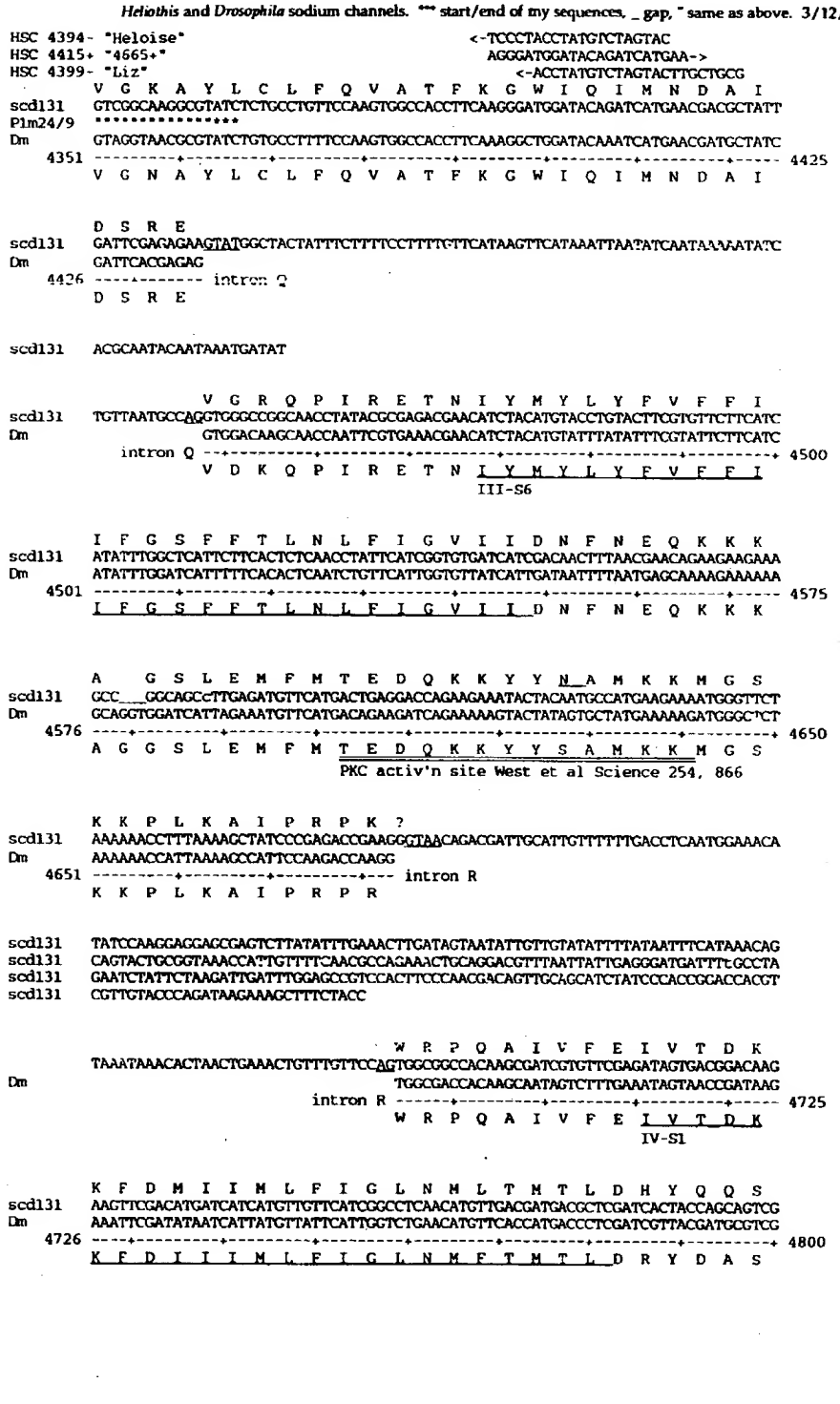

Heliothis and Drosophila sodium channels. *** start/end of my sequences, _ gap, " same as above. 3/12/92 p7.
HSC 4394- "Heloise"
<-TCCCTACCTATGTCTAGTAC
HSC 4415+ "4665+"
AGGGATGGATACAGATCATGAA->
HSC 4399- "Liz"
<-ACCTATGTCTAGTACTTGCTGCG
V G K A Y L C L F Q V A T F K G W I Q I M N D A I
scd131 GTCGGCAAGGCGTATCTCTGCCTGTTCCAAGTGGCCACCTTCAAGGGATGGATACAGATCATGAACGACGCTATT
P1m24/9 ***************
Dm GTAGGTAACGCGTATCTGTGCCTTTTCCAAGTGGCCACCTTCAAAGGCTGGATACAAATCATGAACGATGCTATC
4351 ---------+---------+---------+---------+---------+---------+---------+----- 4425
V G N A Y L C L F Q V A T F K G W I Q I M N D A I
D S R E
scd131 GATTCGAGAGAAGTATGGCTACTATTTCTTTTCCTTTTGTTCATAAGTTCATAAATTAATATCAATAAAAATATC
Dm GATTCACGAGAG
4426 ----+-------- intron Q
D S R E
scd131 ACGCAATACAATAAATGATAT
V G R Q P I R E T N I Y M Y L Y F V F F I
scd131 TGTTAATGCCAGGTGGGCCGGCAACCTATACGCGAGACGAACATCTACATGTACCTGTACTTCGTGTTCTTCATC
Dm GTGGACAAGCAACCAATTCGTGAAACGAACATCTACATGTATTTATATTTCGTATTCTTCATC
intron Q --+---------+---------+---------+---------+---------+---------+---------+ 4500
V D K Q P I R E T N I Y M Y L Y F V F F I
III-S6
I F G S F F T L N L F I G V I I D N F N E Q K K K
scd131 ATATTTGGCTCATTCTTCACTCTCAACCTATTCATCGGTGTGATCATCGACAACTTTAACGAACAGAAGAAGAAA
Dm ATATTTGGATCATTTTTCACACTCAATCTGTTCATTGGTGTTATCATTGATAATTTTAATGAGCAAAAGAAAAAA
4501 ---------+---------+---------+---------+---------+---------+---------+----- 4575
I F G S F F T L N L F I G V I I D N F N E Q K K K
A G S L E M F M T E D Q K K Y Y N A M K K M G S
scd131 GCC GGCAGCcTTGAGATGTTCATGACTGAGGACCAGAAGAAATACTACAATGCCATGAAGAAAATGGGTTCT
Dm GCAGGTGGATCATTAGAAATGTTCATGACAGAAGATCAGAAAAAGTACTATAGTGCTATGAAAAAGATGGGCTCT
4576 ----+---------+---------+---------+---------+---------+---------+---------+ 4650
A G G S L E M F M T E D Q K K Y Y S A M K K M G S
PKC activ'n site West et al Science 254, 866
K K P L K A I P R P K ?
scd131 AAAAAACCTTTAAAAGCTATCCCGAGACCGAAGGGTAACAGACGATTGCATTGTTTTTTGACCTCAATGGAAACA
Dm AAAAAACCATTAAAAGCCATTCCAAGACCAAGG
4651 ---------+---------+---------+--- intron R
K K P L K A I P R P R
scd131 TATCCAAGGAGGAGCGAGTCTTATATTTGAAACTTGATAGTAATATTGTTGTATATTTTATAATTTCATAAACAG
scd131 CAGTACTGCGGTAAACCATTGTTTTCAACGCCAGAAACTGCAGGACGTTTAATTATTGAGGGATGATTTCGCCTA
scd131 GAATCTATTCTAAGATTGATTTGGAGCCGTCCACTTCCCAACGACAGTTGCAGCATCTATCCCACCGCACCACGT
scd131 CGTTGTACCCAGATAAGAAAGCTTTCTACC
W R P Q A I V F E I V T D K
TAAATAAACACTAACTGAAACTGTTTGTTCCAGTGGCGGCCACAAGCGATCGTGTTCGAGATAGTGACGGACAAG
Dm TGGCGACCACAAGCAATAGTCTTTGAAATAGTAACCGATAAG
intron R ------+---------+---------+---------+----- 4725
W R P Q A I V F E I V T D K
IV-S1
K F D M I I M L F I G L N M L T M T L D H Y Q Q S
scd131 AAGTTCGACATGATCATCATGTTGTTCATCGGCCTCAACATGTTGACGATGACGCTCGATCACTACCAGCAGTCG
Dm AAATTCGATATAATCATTATGTTATTCATTGGTCTGAACATGTTCACCATGACCCTCGATCGTTACGATGCGTCG
4726 ----+---------+---------+---------+---------+---------+---------+---------+ 4800
K F D I I I M L F I G L N M F T M T L D R Y D A S

FIGURE 1

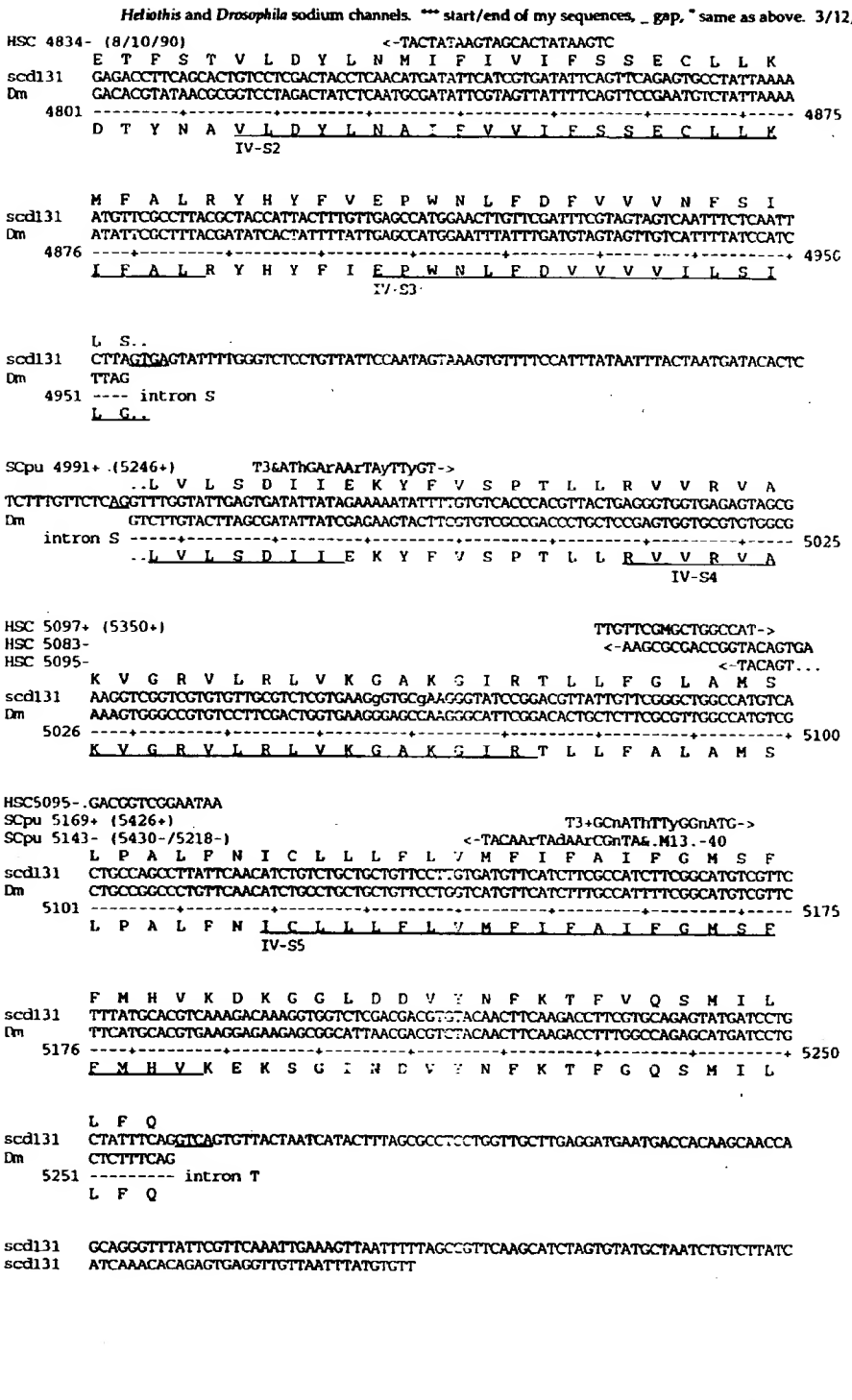

Heliothis and Drosophila sodium channels. *** start/end of my sequences, _ gap, " same as above. 3/12/92 p8.
HSC 4834- (8/10/90)
<-TACTATAAGTAGCACTATAAGTC
E T F S T V L D Y L N M I F I V I F S S E C L L K
scd131 GAGACCTTCAGCACTGTCCTCGACTACCTCAACATGATATTCATCGTGATATTCAGTTCAGAGTGCCTATTAAAA
Dm GACACGTATAACGCGGTCCTAGACTATCTCAATGCGATATTCGTAGTTATTTTCAGTTCCGAATGTCTATTAAAA
4801 4875
D T Y N A V L D Y L N A I F V V I F S S E C L L K
IV-S2
M F A L R Y H Y F V E P W N L F D F V V V N F S I
scd131 ATGTTCGCCTTACGCTACCATTACTTTGTTGAGCCATGGAACTTGTTCGATTTCGTAGTAGTCAATTTCTCAATT
Dm ATATTCGCTTTACGATATCACTATTTTATTGAGCCATGGAATTTATTTGATGTAGTAGTTGTCATTTTATCCATC
4876 4950
I F A L R Y H Y F I E P W N L F D V V V V I L S I
IV-S3
L S..
scd131 CTTAGTGAGTATTTTGGGTCTCCTGTTATTCCAATAGTAAAGTGTTTTCCATTTATAATTTACTAATGATACACTC
Dm TTAG
4951 ---- intron S
L G..
SCpu 4991+ .(5246+)
T3&AThGArAArTAyTTyGT->
..L V L S D I I E K Y F V S P T L L R V V R V A
scd131 TCTTTGTTCTCAGGTTTGGTATTGAGTGATATTATAGAAAAATATTTTGTGTCACCCACGTTACTGAGGGTGGTGAGAGTAGCG
Dm GTCTTGTACTTAGCGATATTATCGAGAAGTACTTCGTGTCGCCGACCCTGCTCCGAGTGGTGCGTGTGGCG
intron S 5025
..L V L S D I I E K Y F V S P T L L R V V R V A
IV-S4
HSC 5097+ (5350+)
HSC 5083-
HSC 5095-
TTGTTCGMGCTGGCCAT->
<-AAGCGCGACCGGTACAGTGA
<-TACAGT...
K V G R V L R L V K G A K G I R T L L F G L A M S
scd131 AAGGTCGGTCGTGTGTTGCGTCTCGTGAAGgGTGCgAAGGGTATCCGGACGTTATTGTTCGGGCTGGCCATGTCA
Dm AAAGTGGGCCGTGTCCTTCGACTGGTGAAGGGAGCCAAGGGCATTCGGACACTGCTCTTCGCGTTGGCCATGTCG
5026 5100
K V G R V L R L V K G A K G I R T L L F A L A M S
HSC5095-.GACGGTCGGAATAA
SCpu 5169+ (5426+)
T3+GCnAThTTyGGnATG->
SCpu 5143- (5430-/5218-)
<-TACAArTAdAArCGnTA&.M13.-40
L P A L F N I C L L L F L V M F I F A I F G M S F
scd131 CTGCCAGCCTTATTCAACATCTGTCTGCTGCTGTTCCTTGTGATGTTCATCTTCGCCATCTTCGGCATGTCGTTC
Dm CTGCCGGCCCTGTTCAACATCTGCCTGCTGCTGTTCCTGGTCATGTTCATCTTTGCCATTTTCGGCATGTCGTTC
5101 5175
L P A L F N I C L L L F L V M F I F A I F G M S F
IV-S5
F M H V K D K G G L D D V Y N F K T F V Q S M I L
scd131 TTTATGCACGTCAAAGACAAAGGTGGTCTCGACGACGTGTACAACTTCAAGACCTTCGTGCAGAGTATGATCCTG
Dm TTCATGCACGTGAAGGAGAAGAGCGGCATTAACGACGTCTACAACTTCAAGACCTTTGGCCAGAGCATGATCCTG
5176 5250
F M H V K E K S G I N D V Y N F K T F G Q S M I L
L F Q
scd131 CTATTTCAGGTCAGTGTTACTAATCATACTTTAGCGCCTCCTGGTTGCTTGAGGATGAATGACCACAAGCAACCA
Dm CTCTTTCAG
5251 --------- intron T
L F Q
scd131 GCAGGGTTTATTCGTTCAAATTGAAAGTTAATTTTTAGCCGTTCAAGCATCTAGTGTATGCTAATCTGTCTTATC
scd131 ATCAAACACAGAGTGAGGTTGTTAATTTATGTGTT

FIGURE 1

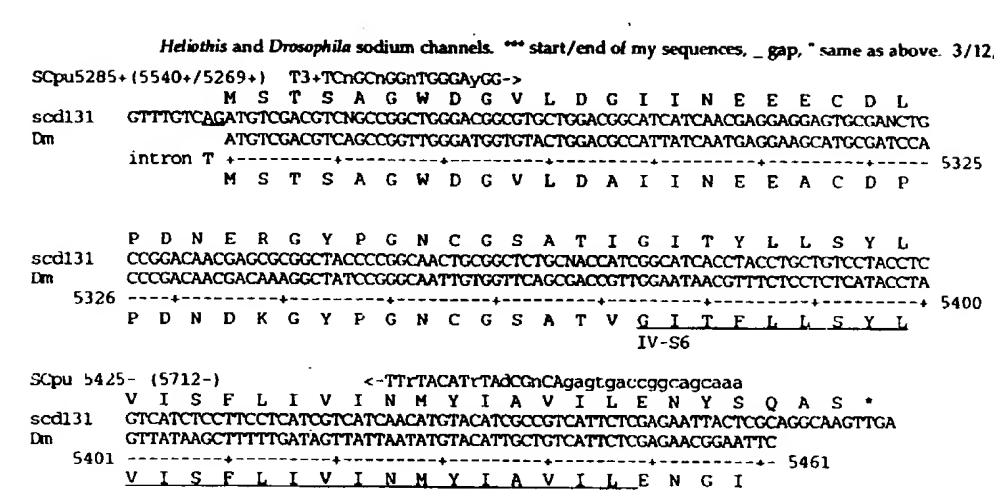
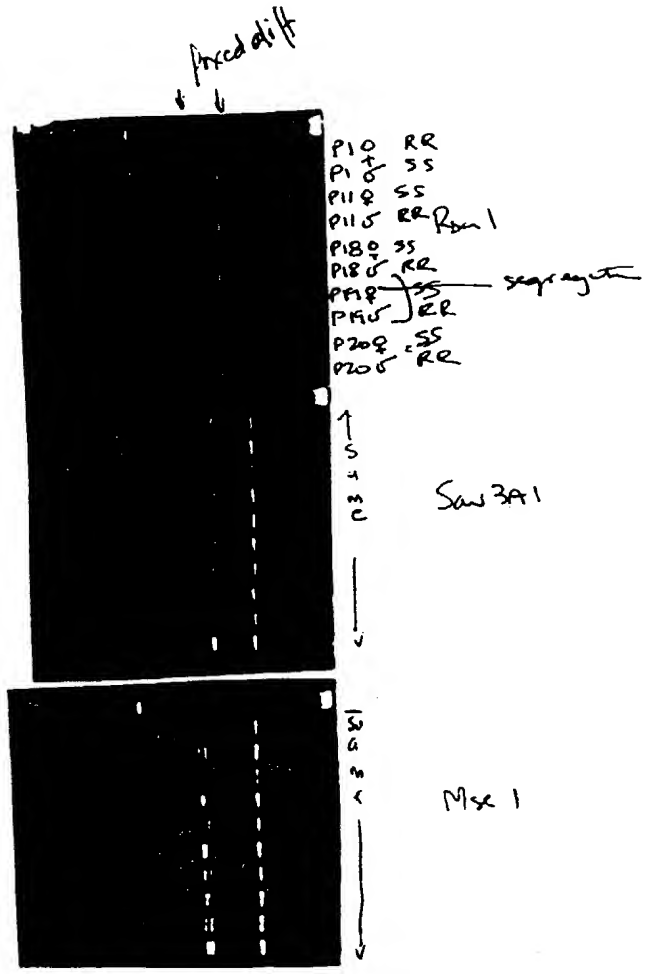

Heliothis and Drosophila sodium channels. *** start/end of my sequences, _ gap, " same as above. 3/12/92 p9.
SCpu5285+(5540+/5269+) T3+TCnGCnGGnTGGGAyGG->
M S T S A G W D G V L D G I I N E E E C D L
scd131 GTTTGTCAGATGTCGACGTCNGCCGGCTGGGACGGCGTGCTGGACGGCATCATCAACGAGGAGGAGTGCGANCTG
Dm ATGTCGACGTCAGCCGGTTGGGATGGTGTACTGGACGCCATTATCAATGAGGAAGCATGCGATCCA
intron T +---------+---------+---------+---------+---------+---------+----- 5325
M S T S A G W D G V L D A I I N E E A C D P
P D N E R G Y P G N C G S A T I G I T Y L L S Y L
scd131 CCGGACAACGAGCGCGGCTACCCCGGCAACTGCGGCTCTGCNACCATCGGCATCACCTACCTGCTGTCCTACCTC
Dm CCCGACAACGACAAAGGCTATCCGGGCAATTGTGGTTCAGCGACCGTTGGAATAACGTTTCTCCTCTCATACCTA
5326 ----+---------+---------+---------+---------+---------+---------+---------+ 5400
P D N D K G Y P G N C G S A T V G I T F L L S Y L
IV-S6
SCpu 5425- (5712-) <-TTrTACATrTAdCGnCAgagtgaccggcagcaaa
V I S F L I V I N M Y I A V I L E N Y S Q A S *
scd131 GTCATCTCCTTCCTCATCGTCATCAACATGTACATCGCCGTCATTCTCGAGAATTACTCGCAGGCAAGTTGA
Dm GTTATAAGCTTTTTGATAGTTATTAATATGTACATTGCTGTCATTCTCGAGAACGGAATTC
5401 ---------+---------+---------+---------+---------+---------+- 5461
V I S F L I V I N M Y I A V I L E N G I

FIGURE 2


forced diff
P10 RR
P11 SS
P11 RR
P18 SS
P18 RR
P19 SS
P19 RR
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P20 RR
segregating
Sau 3A1
Msx 1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: American Cyanamid Company

(ii) TITLE OF INVENTION: Method for Monitoring Pesticide Resistance

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: American Cyanamid Company
(B) STREET: One Cyanamid Plaza
(C) CITY: Wayne
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) ZIP: 07470-8426

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: EP 93 118 061.6
(B) FILING DATE: 08-NOV-1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Wachtershauser Dr., Gunter
(C) REFERENCE/DOCKET NUMBER: EA-9088/31,732

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (089)293906
(B) TELEFAX: (089)223759
(C) TELEX: 5214173 Patw-D

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2416 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AATTCACTAT ACCAGGTAAC TTTTTGATAC CTAGTTTAAA ATAAGATACT GTTGTTATCT 60

AATAGGATTT TAAGAGTTGT CATAAACGTA ATGTTAATTT TTCAGGCGAC AATAAATACA 120

AGAAAGGGCA AAATTTTGTT AAATAATATT AACGCAWTAA CAGATAATCA TAGAGACAAC 180

CGTTTAGACT GTGAATTAAA TCATCACGGG TATCCTATAC AGGTAAATAT TTGTCGTCAC 240

AGCTTKCTAA TAAATCACAA TCAAGTTTCT GTACTAAGAA CACAATTTCT CGTTTAGGAT 300

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GACGATACAA TTAGTCAAAA ATCGTACGGC AGTCATAAAA TCAGGTCGTT CAAAGATGAA   360
AGTCATAAAG GTTCCGCAGA CACGATAGAT GGCGAMGMGM MGAAGGACGC TAGTAAAGAG   420
GAATTGGGTT TAGAAGAAGG TCAGTGTAAA ACTGCAATTN AAAATTAACA GAATTGAACT   480
AAGCCATATT TGGACAATTT GCATATAATT AATGTGTTAC AGAAATGGTT GAAGAAGAGG   540
AAGATGGGAA GTTAGACGGA GGTCTAGGCA AAACAGACAT TATAGTGGCC GCAGATGAAG   600
AAGTTGTTGA CGATAGCCCT GCTGACTGCT GTCCAGAGCC ATGTTACGCG AAGTTTCCAT   660
TCCTTGTGGG TGATGATGAA TCTCCCTTTT GGCAAGGCTG GGGCATGCTT CGGTTGAAAA   720
CCTTCAAACT CATTGAGAAC ACATATTTCG AAACGGCTGT GATTACAATG ATTTTGCTCA   780
GTAGTTTGGC TTTGGTAAGT TCTCAAATAA TTTTCTGAAC ACTTTGTTTC ACATAGTAAG   840
GGAGCAAATT ATGTTCATGA CGAAACTTYK CTGTCTTTAC AGGCTTTAGA AGATGTAAAT   900
TTACCACATC GACCGATTCT TCAAGATATC TTGTATTATA TGGATCGGAT CTTCACCGTC   960
ATTTTCTTCA TCGAGATGTT GATCAAATGG CTTGCCCTTG GCTTCCAGAA ATACTTCACA  1020
AATGCGTGGT GCTGGCTCGA CTTCATCATT GTCATGGTAA TATTACTATA AATATATTTG  1080
CTTTCGTATC ATTTGAACTA ACAGTTTCCT TGCAGATTAG ATTGGTAAAA CGTAGATTAT  1140
GATTATGGAA TTTGAACTTG TAAGTTCTGT ATAATGTGAA AGACAAAATT AAGGTTCAGG  1200
TCGGTCTTTG AAGTTTATCC TGCCGCCTCT CAGCGAGGTA AAGCTGGGAA GAATAATTTA  1260
TACAGTGTTA AGTATACCTA GATGTAAGGA ATATATTGTA TACTAAAGTA AATGACGATT  1320
GGTGTGGCGT TAGTTGTCGC TCGTAAACCA CGGNGCAGTG ATGSTGGCGS GACGACATCC  1380
CNGTTCCGCT CGATGCACGT TGNGNGCGCT GCGGCTCCGC GCGGTCTCTC GCTGGGAGGG  1440
CATGCGCGTG AGTAGGACGG CACACCACTC GTGCGCAGGC TGTGTTGGTA TCGTTGCGCT  1500
GCACATCCAC ACGATTGTTT CACTCTACTT TCTGCTGAGA AATCAGTGCA ACATGGTGTT  1560
GCTAATCGAA ATAAGCAACC AAACCTTCCG ACAGAGATTT TTATCTCGAA CCACTTTGTG  1620
AAATGTGAAC TCTGATTCAT ATTCAACTAA TCTCTTAATA AAGTTTGTTG TAAATATTTT  1680
CTAATTCTAC TGTGTTTGAC GTGCAGCGCA ACTCAAAGCG TGCAGCTTTG ATTGTTCGAT  1740
GTCTATGGCA GTGGAAACTC CGAACGGCCT CACCTCGCTG CCTCGAGCTC TCGATGTCGT  1800
ATTGTTTGTT TATGGAAACC GCTTCATGTG ACTCTATAAC CCACGACCCC CGCTATATGA  1860
ATACCTGTGR CCGTATATAT AAAAACCTCC ACAGAGTGAC TTGAAATCCT TATACTTTCA  1920
AGTGCATGAA ACAACACGTC TTCTATCTTT GTGCTGTTGT GCGAGATAGT GCGTTTTCAC  1980
GTACTACTCA CATTACCCAC ATCTGTCGGG GATAAAATCC GASATTTGAA AGAAAAGCTT  2040
TAAAACTGAA AATGGCACGT GATGTTGGTT GCTGTCGATG TCATTACAAA GCAAACTATA  2100
AATACCTATA CTATATACAT ATCTTTGATA TTTGTTCTTA ATATGATGTG ATGTAGCTTT  2160
ATTTTAGGGA CATCAGAGAA ACGGTAGCCT AAGCTCAAAA TTAGAGCTTT TTGTAAAATC  2220
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AATCCTGTTA ATTGCTATAT AATTATTTCC ATTTCTTTTA TTCTCTGATG KYCYYMAARK 2280

WAMYTCGATG TAACCTTATG TGTAACTTGA GTGAATATCA CGTTCCTATC CCTCTGATTA 2340

TGCTGCAATA GGAACTTCTG TTTCCAAATG AATCTTGAGA TTTTCTTCTT TATAGTATCA 2400

TATCCTTAGG TTTGTA 2416

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 567 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATTAGCGTTC AAAAGCGATG CGAAGCTGGG ACTGCGCTCT CAGGCCATGA GCCGCATGCA 60

GGGCATGAGG GTACGTACCA CCCTGTGCTG CCGACAACAC CCTATCGCTC ATCCATCCAC 120

CACACACTTC GCTCCACACT TCACATTCAC ATTTCTATTT CAACTTCTAC GATCATTTTT 180

TAACATTTTA AAATTTCCAA CGTRCCAGCC GTACTMGGGC TCCTTTTTTC GATATTTCTG 240

CATSAATCAC CGGATCAAAA TTTGTTTTTA ATAGTTAATT TGGACAGTTA TCCGATTCAT 300

TGGCAGTAGT CGATTGAAGT AATTATTAGT GAATCATTTT GAAGTGGTCG GTGGCACCCC 360

TGAATGGCTT AGTATCATCA CTGTTCGTCA TAAACCTCTT TTAGAAAGGG TCAATGGGAT 420

TTATTGTGGA GAGATATTYR TCCATGTTTT GGTCTCTTTT CTATTGGTCT TATTATTAGC 480

TAGATTAGAC TTTTGTAATT ACTTAGTTAT TTGGAATGCT AATTTATATT CTGCACCTTA 540

GATTTTTTCT TCTTGTATCT TCATCGA 567

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCTAACTGCT ACATAGTTAC TGCACAGTAT TAATGACATT AACGTCCTTA TATCCCAACT 60

AATAATGCGC CCACTAACAA ATGCACGCCA TTGATATAAG AAAGGAGACG TATCAGTACT 120

TCCAATATAT CCTTCGTGAC CAGTGTAGTA ATACGTACGT ATGTGACAGG TGGTGGTAAA 180

CGCTCTCGTG CAAGCGATCC CGTCCATCTT CAACGTGTTG TTGGTGTGTC TTATCTTCTG 240

GCTGATCTTC GCCATCATGG GAGTACAACT GTTCGCTGGC AAATATTTCA AGGTATTAAT 300

TTATTAACAT AACAAAAAAA TATTTCAATT CGTAAAATCT TATTAGTGTG TTCAAAATTT 360

CTAACATGTT TTTCTTTGTT CTGTTCTAGT GCGTCGACCT CAACCACACG ACGTTGAGCC 420

ACGAAATCAT CCCAGACCGG AATGCGTGCA TCTTAGAGAA CTACACCTGG GAGAACTCAC 480

CGATGAACTT TGACCATGTC GGCAAGGCGT ATCTCTGCCT GTTCCAAGTG GCCACCTTCA 540

AGGGATGGAT ACAGATCATG AACGACGCTA TTGATTCGAG AGAAGTATGG CTACTATTTC 600

TTTTCCTTTT GTTCATAAGT TCATAAATTA ATATCAATAA AAATATCACG CAATACAATA 660

AATGATATTG TTAATGCCAG GTGGGCCGGC AACCTATACG CGAGACGAAC ATCTACATGT 720

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GTGTGATCAT CGACAACTTT AACGAACAGA AGAAGAAAGC CGGCGGCAGC CTTGAGATGT 840

TCATGACTGA GGACCAGAAG AAATACTACA ATGCCATGAA GAAAATGGGT TCTAAAAAAC 900

CTTTAAAAGC TATCCCGAGA CCGAAGGTAA CAGACGATTG CATTGTTTTT TGACCTCAAT 960

GGAAACATAT CCAAGGAGGA GCGAGTCTTA TATTTGAAAC TTGATAGTAA TATTGTTGTA 1020

TATTTTATAA TTTCATAAAC AGCAGTACTG CGGTAAACCA TTGTTTTCAA CGCCAGAAAC 1080

TGCAGGACGT TTAATTATTG AGGGATGATT TTGCCTAGAA TCTATTCTAA GATTGATTTG 1140

GAGCCGTCCA CTTCCCAACG ACAGTTGCAG CATCTATGCC ACCGGACCAC GTCGTTGTAC 1200

CCAGATAAGA AAGCTTTCTA CCTAAATAAA CACTAACTGA AACTGTTTGT TCCAGTGGCG 1260

GCCACAAGCG ATCGTGTTCG AGATAGTGAC GGACAAGAAG TTCGACATGA TCATCATGTT 1320

GTTCATCGGC CTCAACATGT TGACGATGAC GCTCGATCAC TACCAGCAGT CGGAGACCTT 1380

CAGCACTGTC CTCGACTACC TCAACATGAT ATTCATCGTG ATATTCAGTT CAGAGTGCCT 1440

ATTAAAAATG TTCGCCTTAC GCTACCATTA CTTTGTTGAG CCATGGAACT TGTTCGATTT 1500

CGTAGTAGTC AATTTCTCAA TTCTTAGTGA GTATTTTGGG TCTCCTGTTA TTCCAATAGT 1560

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TGAGTGATAT TATAGAAAAA TATTTTGTGT CACCCACGTT ACTGAGGGTG GTGAGAGTAG 1680

CGAAGGTCGG TCGTGTGTTG CGTCTCGTGA AGGGTGCGAA GGGTATCCGG ACGTTATTGT 1740

TCGGGCTGGC CATGTCACTG CCAGCCTTAT TCAACATCTG TCTGCTGCTG TTCCTTGTGA 1800

TGTTCATCTT CGCCATCTTC GGCATGTCGT TCTTTATGCA CGTCAAAGAC AAAGGTGGTC 1860

TCGACGACGT GTACAACTTC AAGACCTTCG TGCAGAGTAT GATCCTGCTA TTTCAGGTCA 1920

GTGTTACTAA TCATACTTTA GCGCCTCCTG GTTGCTTGAG GATGAATGAC CACAAGCAAC 1980

CAGCAGGGTT TATTCGTTCA AATTGAAAGT TAATTTTTAG CCGTTCAAGC ATCTAGTGTA 2040

TGCTAATCTG TCTTATCGTT TGTCAGATGT CGACGTCNGC CGGCTGGGAC GGCGTGCTGG 2100

ACGGCATCAT CAACGAGGAG GAGTGCGANC TGCCGGACAA CGAGCGCGGC TACCCCGGCA 2160

ACTGCGGCTC TGCNACCATC GGCATCACCT ACCTGCTGTC CTACCTCGTC ATCTCCTTCC 2220

TCATCGTCAT CAACATGTAC ATCGCCGTCA TTCTCGAGAA TTACTCGCAG GCAAGTTGA 2279

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 196 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asp Asp Asp Thr Ile Ser Gln Lys Ser Tyr Gly Ser His Lys Ile Arg
1 5 10 15

Ser Phe Lys Asp Glu Ser His Lys Gly Ser Ala Asp Thr Ile Asp Gly
20 25 30

Xaa Xaa Xaa Lys Asp Ala Ser Lys Glu Glu Leu Gly Leu Glu Glu Glu
35 40 45

Met Val Glu Glu Glu Glu Asp Gly Lys Leu Asp Gly Gly Leu Gly Lys
50 55 60

Thr Asp Ile Ile Val Ala Ala Asp Glu Glu Val Val Asp Asp Ser Pro
65 70 75 80

Ala Asp Cys Cys Pro Glu Pro Cys Tyr Ala Lys Phe Pro Phe Leu Val
85 90 95

Gly Asp Asp Glu Ser Pro Phe Trp Gln Gly Trp Gly Met Leu Arg Leu
100 105 110

Lys Thr Phe Lys Leu Ile Glu Asn Thr Tyr Phe Glu Thr Ala Val Ile
115 120 125

Thr Met Ile Leu Leu Ser Ser Leu Ala Leu Ala Leu Glu Asp Val Asn
130 135 140

Leu Pro His Arg Pro Ile Leu Gln Asp Ile Leu Tyr Tyr Met Asp Arg
145 150 155 160

Ile Phe Thr Val Ile Phe Phe Ile Glu Met Leu Ile Lys Trp Leu Ala
165 170 175

Leu Gly Phe Gln Lys Tyr Phe Thr Asn Ala Trp Cys Trp Leu Asp Phe
180 185 190

Ile Ile Val Met
195

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Met Ser Arg Met Gln Gly Met Arg
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 452 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Val Val Val Asn Ala Leu Val Gln Ala Ile Pro Ser Ile Phe Asn Val
1 5 10 15

Leu Leu Val Cys Leu Ile Phe Trp Leu Ile Phe Ala Ile Met Gly Val
20 25 30

Gln Leu Phe Ala Gly Lys Tyr Phe Lys Cys Val Asp Leu Asn His Thr
35 40 45

Thr Leu Ser His Glu Ile Ile Pro Asp Arg Asn Ala Cys Ile Leu Glu
50 55 60

Asn Tyr Thr Trp Glu Asn Ser Pro Met Asn Phe Asp His Val Gly Lys
65 70 75 80

Ala Tyr Leu Cys Leu Phe Gln Val Ala Thr Phe Lys Gly Trp Ile Gln
85 90 95

Ile Met Asn Asp Ala Ile Asp Ser Arg Glu Val Gly Arg Gln Pro Ile
100 105 110

Arg Glu Thr Asn Ile Tyr Met Tyr Leu Tyr Phe Val Phe Phe Ile Ile
115 120 125

Phe Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile Gly Val Ile Ile Asp
130 135 140

Asn Phe Asn Glu Gln Lys Lys Lys Ala Ala Gly Ser Leu Glu Met Phe
145 150 155 160

Met Thr Glu Asp Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys Met Gly
165 170 175

Ser Lys Lys Pro Leu Lys Ala Ile Pro Arg Pro Lys Trp Arg Pro Gln
180 185 190

Ala Ile Val Phe Glu Ile Val Thr Asp Lys Lys Phe Asp Met Ile Ile
195 200 205

Met Leu Phe Ile Gly Leu Asn Met Leu Thr Met Thr Leu Asp His Tyr
210 215 220

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Gln Gln Ser Glu Thr Phe Ser Thr Val Leu Asp Tyr Leu Asn Met Ile
225                 230                 235                 240

Phe Ile Val Ile Phe Ser Ser Glu Cys Leu Leu Lys Met Phe Ala Leu
                245                 250                 255

Arg Tyr His Tyr Phe Val Glu Pro Trp Asn Leu Phe Asp Phe Val Val
            260                 265                 270

Val Asn Phe Ser Ile Leu Ser Leu Val Leu Ser Asp Ile Ile Glu Lys
        275                 280                 285

Tyr Phe Val Ser Pro Thr Leu Leu Arg Val Val Arg Val Ala Lys Val
    290                 295                 300

Gly Arg Val Leu Arg Leu Val Lys Gly Ala Lys Gly Ile Arg Thr Leu
305                 310                 315                 320

Leu Phe Gly Leu Ala Met Ser Leu Pro Ala Leu Phe Asn Ile Cys Leu
                325                 330                 335

Leu Leu Phe Leu Val Met Phe Ile Phe Ala Ile Phe Gly Met Ser Phe
            340                 345                 350

Phe Met His Val Lys Asp Lys Gly Gly Leu Asp Asp Val Tyr Asn Phe
        355                 360                 365

Lys Thr Phe Val Gln Ser Met Ile Leu Leu Phe Gln Met Ser Thr Ser
    370                 375                 380

Ala Gly Trp Asp Gly Val Leu Asp Gly Ile Ile Asn Glu Glu Glu Cys
385                 390                 395                 400

Asp Leu Pro Asp Asn Glu Arg Gly Tyr Pro Gly Asn Cys Gly Ser Ala
                405                 410                 415

Thr Ile Gly Ile Thr Tyr Leu Leu Ser Tyr Leu Ala Ala Val Ile Ser
            420                 425                 430

Phe Leu Ile Val Ile Asn Met Tyr Ile Ala Val Ile Leu Glu Asn Tyr
        435                 440                 445

Ser Gln Ala Ser
    450
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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5461 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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ATGACAGAAG ATTCCGACTC GATATCTGAG GAAGAACGCA GTTTGTTCCG TCCCTTTACC      60

CGCGAATCAT TGGTGCAAAT CGAACAACGC ATTGCCGCTG AACATGAAAA GCAGAAGGAG     120

CTGGAAAGAA AGAGAGCCGA GGGAGAGGTG CCGCGATATG GTCGCAAGAA AAAACAAAAA     180
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GAAATCCGAT ATGATGACGA GGACGAGGAT GAAGGTCCAC AACCGGATCC TACACTTGAA    240
CAGGGTGTGC CAATACCTGT TCGATTGCAG GGCAGCTTCC CGCCGGAATT GGCCTCCACT    300
CCTCTCGAGG ATATCGATCC CTACTACAGC AATGTACTGA CATTCGTAGT TGTAAGCAAA    360
GGAAAAGATA TTTTTCGCTT TTCTGCATCA AAAGCAATGT GGATGCTCGA TCCATTCAAT    420
CCGATACGTC GTGTGGCCAT TTACATTCTA GTGCATCCAT TATTTTCCCT ATTCATCATC    480
ACCACAATTC TCGTCAACTG CATCCTGATG ATAATGCCGA CAACGCCCAC GGTTGAGTCC    540
ACTGAGGTGA TATTCACCGG AATCTACACA TTTGAATCAG CTGTTAAAGT GATGGCACGA    600
GGTTTCATTT TATGCCCGTT TACGTATCTT AGAGATGCAT GGAATTGGCT GGACTTCGTA    660
GTAATAGCTT TAGCTTATGT GACCATGGGT ATAGATTTAG GTAATCTAGC AGCCCTGCGA    720
ACGTTTAGGG TGCTGCGAGC GCTTAAAACC GTAGCCATTG TGCCAGGCTT GAAGACCATC    780
GTCGGCGCCG TCATCGAATC GGTGAAGAAT CTGCGCGATG TGATTATCCT GACCATGTTC    840
TCCCTGTCGG TGTTCGCGTT GATGGGCCTA CAGATCTATA TGGGCGTGCT CACCGAGAAG    900
TGCATCAAGA AGTTCCCGCT GGACGGTTCC TGGGGCAATC TGACCGACGA GAACTGGGAC    960
TATCACAATC GCAATAGCTC CAATTGGTAT TCCGAGGACG AGGGCATCTC ATTTCCGTTA   1020
TGCGGCAATA TATCCGGTGC GGGGCAATGC GACGACGATT ACGTGTGCCT GCAGGGGTTT   1080
GGTCCGAATC CGAATTATGG CTACACCAGC TTCGATTCGT TCGGATGGGC TTTCCTGTCC   1140
GCCTTCCGGC TGATGACACA GGACTTCTGG GAGGATCTGT ACCAGCTGGT GTTGCGCGCC   1200
GCCGGACCAT GGCACATGCT GTTCTTTATA GTCATCATCT TCCTAGGTTC ATTCTATCTT   1260
GTGAATTTGA TTTTGGCCAT TGTTGCCATG TCGTATGACG AATTGCAAAG GAAGGCCGAA   1320
GAAGAAGAGG CTGCCGAAGA GGAGGCGATA CGTGAAGCGG AAGAAGCTGC CGCCGCCAAA   1380
GCGGCCAAGC TGGAGGAGCG GGCCAATGCG CAGGCTCAGG CAGCAGCGGA TGCGGCTGCC   1440
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TATGAGCTAT TTGTTGGCGG CGAGAAGGGC AACGATGACA ACAACAAAGA GAAGATGTCC   1560
ATTCGGAGCG TCGAGGTGGA GTCGGAGTCG GTGAGCGTTA TACAAAGACA ACCAGCACCT   1620
ACCACAGCAC ACCAAGCTAC CAAAGTTCGT AAAGTGAGCA CGTACACGAT ACGGAACGGA   1680
CGTGGCCGCT TTGGTATACC CGGTAGCGAT CGTAAGCCAT TGGTATTGTC AACATATCAG   1740
GATGCCCAGC AGCACTTGCC CTATGCCGAC GACTCGAATG CCGTCACCCC GATGTCCGAA   1800
GAGAATGGGG CCATCATAGT GCCCGTGTAC TATGGCAATC TAGGCTCCCG ACACTCATCG   1860
TATACCTCGC ATCAGTCCCG AATATCGTAT ACCTCACATG GCGATCTACT CGGCGGCATG   1920
GCCGTCATGG GCGTCAGCAC AATGACCAAG GAGAGCAAAT TGCGCAACCG CAACACACGC   1980
AATCAATCAG TGGGCGCCAC CAATGGCGGC ACCACCTGTC TGGACACCAA TCACAAGCTC   2040
GATCATCGCG ACTACGAAAT TGGCCTGGAG TGCACGGACG AAGCTGGCAA GATTAAACAT   2100
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CATGACAATC CTTTTATCGA GCCCGTCCAG ACACAAACGG TGGTTGATAT GAAAGATGTG   2160
ATGGTCCTGA ATGACATCAT CGAACAGGCC GCTGGTCGGC ACAGTCGGGC AAGCGATCGC   2220
GGTGTCTCCG TTTACTATTT CCCAACAGAG GACGATGACG AGGATGGGCC GACGTTCAAA   2280
GACAAGGCAC TCGAAGTGAT CCTCAAAGGC ATCGATGTGT TTTGTGTGTG GGACTGTTGC   2340
TGGGTTTGGT TGAAATTTCA GGAGTGGGTA TCGCTCATCG TCTTCGATCC CTTCGTCGAG   2400
CTCTTCATCA CGCTGTGCAT TGTGGTCAAC ACGATGTTCA TGGCAATGGA TCACCACGAT   2460
ATGAACAAGG AGATGGAACG CGTGCTCAAG AGTGGCAACT ATTTCTTCAC CGCCACCTTT   2520
GCCATCGAGG CCACCATGAA GCTAATGGCC ATGAGCCCCA AGTACTATTT CCAGGAGGGC   2580
TGGAACATCT TCGACTTCAT TATCGTGGCC CTATCGCTAT TGGAACTGGG ACTCGAGGGT   2640
GTCCAGGGTC TGTCCGTATT GCGTTCCTTT CGATTGCTGC GTGTATTCAA ACTGGCCAAG   2700
TCTTGGCCCA CACTTAATTT ACTCATTTCG ATTATGGGAC GCACCATGGG CGCTTTGGGT   2760
AATCTGACAT TTGTACTTTG CATTATCATC TTCATCTTTG CGGTGATGGG AATGCAACTG   2820
TTCGGAAAGA ATTATCATGA TCACAAGGAC CGCTTTCCGG ATGGCGACCT GCCGCGCTGG   2880
AACTTCACCG ACTTTATGCA CAGCTTCATG ATCGTGTTCC GGGTGCTCTG CGGAGAATGG   2940
ATCGAGTCCA TGTGGGACTG CATGTACGTG GGCGATGTCT CGTGCATTCC CTTCTTCTTG   3000
GCCACCGTTG TCATCGGCAA TCTTGTGGTA CTTAACCTTT TCTTAGCCTT GCTTTTGTCC   3060
AATTTTGGCT CATCTAGCTT ATCAGCGCCG ACTGCCGATA ACGATACGAA TAAAATAGCC   3120
GAGGCCTTCA ATCGAATTGG CCGATTTAAA AGTTGGGTTA AGCGTAATAT TGCTGATTGT   3180
TTCAAGTTAA TACGTAACAA ATTGACAAAT CAAATAAGTG ATCAACCATC AGAGCATGGT   3240
GACAACGAAC TGGAGCTGGG CCACGACGAG ATCCTCGCCG ACGGCCTCAT CAAGAAGGGG   3300
ATCAAGGAGC AGACGCAACT GGAGGTGGCC ATCGGGGATG GCATGGAATT CACGATACAC   3360
GGCGACATGA AGAACAACAA GCCGAAGAAA TCCAAATATC TAAATAACGC AACGGACGAC   3420
GACACTGCCA GCATTAACTC ATATGGTAGC CATAAGAATC GACCATTCAA GGACGAGAGC   3480
CACAAGGGCA GCGCCGAGAC GATGGAGGGC GAGGAGAAGC GCGACGCCAG CAAGGAGGAT   3540
TTAGGTCTCG ACGAGGAACT GGACGAGGAG GGCGAATGCG AGGAGGGCCC GCTCGACGGT   3600
GATATCATTA TTCATGCACA CGACGAGGAT ATACTCGATG AATATCCAGC TGATTGCTGC   3660
CCCGATTCGT ACTATAAGAA ATTTCCGATC TTAGCCGGTG ACGATGACTC GCCGTTCTGG   3720
CAAGGATGGG GCAATTTACG ACTGAAAACT TTTCGATTAA TTGAGGATAA ATATTTTGAA   3780
ACAGCTGTTA TCACTATGAT TTTAATGAGT AGCTTAGCTT TGGCATTAGA AGATGTACAT   3840
CTGCCACAAA GACCCATACT GCAGGATATT TTATACTATA TGGACAGAAT ATTTACGGTT   3900
ATATTCTTCT TGGAAATGTT AATCAAGTGG TTGGCGCTCG GCTTCAAAGT GTACTTGACC   3960
AACGCGTGGT GTTGGCTCGA TTTCGTGATT GTCATGGTAT CGCTTATCAA CTTCGTTGCT   4020
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TCACTTGTTG GAGCTGGTGG TATTCAAGCC TTCAAGACTA TGCGAACGTT AAGAGCACTG 4080

AGACCACTAC GTGCCATGTC CCGTATGCAG GGCATGAGGG TCGTCGTTAA TGCGCTGGTA 4140

CAAGCTATAC CGTCCATCTT CAATGTGCTA TTGGTGTGTC TAATATTTTG GCTAATTTTT 4200

GCCATAATGG GTGTACAGCT TTTTGCTGGA AAATATTTTA AGTGCGAGGA CATGAATGGC 4260

ACGAAGCTCA GCCACGAGAT CATACCAAAT CGCAATGCCT GCGAGAGCGA GAACTACACG 4320

TGGGTGAATT CAGCAATGAA TTTCGATCAT GTAGGTAACG CGTATCTGTG CCTTTTCCAA 4380

GTGGCCACCT TCAAAGGCTG GATACAAATC ATGAACGATG CTATCGATTC ACGAGAGGTG 4440

GACAAGCAAC CAATTCGTGA AACGAACATC TACATGTATT TATATTTCGT ATTCTTCATC 4500

ATATTTGGAT CATTTTTCAC ACTCAATCTG TTCATTGGTG TTATCATTGA TAATTTTAAT 4560

GAGCAAAAGA AAAAAGCAGG TGGATCATTA GAAATGTTCA TGACAGAAGA TCAGAAAAAG 4620

TACTATAGTG CTATGAAAAA GATGGGCTCT AAAAAACCAT TAAAAGCCAT TCCAAGACCA 4680

AGGTGGCGAC CACAAGCAAT AGTCTTTGAA ATAGTAACCG ATAAGAAATT CGATATAATC 4740

ATTATGTTAT TCATTGGTCT GAACATGTTC ACCATGACCC TCGATCGTTA CGATGCGTCG 4800

GACACGTATA ACGCGGTCCT AGACTATCTC AATGCGATAT TCGTAGTTAT TTTCAGTTCC 4860

GAATGTCTAT TAAAAATATT CGCTTTACGA TATCACTATT TTATTGAGCC ATGGAATTTA 4920

TTTGATGTAG TAGTTGTCAT TTTATCCATC TTAGGTCTTG TACTTAGCGA TATTATCGAG 4980

AAGTACTTCG TGTCGCCGAC CCTGCTCCGA GTGGTGCGTG TGGCGAAAGT GGGCCGTGTC 5040

CTTCGACTGG TGAAGGGAGC CAAGGGCATT CGGACACTGC TCTTCGCGTT GGCCATGTCG 5100

CTGCCGGCCC TGTTCAACAT CTGCCTGCTG CTGTTCCTGG TCATGTTCAT CTTTGCCATT 5160

TTCGGCATGT CGTTCTTCAT GCACGTGAAG GAGAAGAGCG GCATTAACGA CGTCTACAAC 5220

TTCAAGACCT TTGGCCAGAG CATGATCCTG CTCTTTCAGA TGTCGACGTC AGCCGGTTGG 5280

GATGGTGTAC TGGACGCCAT TATCAATGAG GAAGCATGCG ATCCACCCGA CAACGACAAA 5340

GGCTATCCGG GCAATTGTGG TTCAGCGACC GTTGGAATAA CGTTTCTCCT CTCATACCTA 5400

GTTATAAGCT TTTTGATAGT TATTAATATG TACATTGCTG TCATTCTCGA GAACGGAATT 5460

C 5461

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1820 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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Met Thr Glu Asp Ser Asp Ser Ile Ser Glu Glu Glu Arg Ser Leu Phe
1               5                  10                  15

Arg Pro Phe Thr Arg Glu Ser Leu Val Gln Ile Glu Gln Arg Ile Ala
            20                  25                  30

Ala Glu His Glu Lys Gln Lys Glu Leu Glu Arg Lys Arg Ala Glu Gly
        35                  40                  45

Glu Val Pro Arg Tyr Gly Arg Lys Lys Lys Gln Lys Glu Ile Arg Tyr
    50                  55                  60

Asp Asp Glu Asp Glu Asp Glu Gly Pro Gln Pro Asp Pro Thr Leu Glu
65                  70                  75                  80

Gln Gly Val Pro Ile Pro Val Arg Leu Gln Gly Ser Phe Pro Pro Glu
                85                  90                  95

Leu Ala Ser Thr Pro Leu Glu Asp Ile Asp Pro Tyr Tyr Ser Asn Val
            100                 105                 110

Leu Thr Phe Val Val Val Ser Lys Gly Lys Asp Ile Phe Arg Phe Ser
        115                 120                 125

Ala Ser Lys Ala Met Trp Met Leu Asp Pro Phe Asn Pro Ile Arg Arg
    130                 135                 140

Val Ala Ile Tyr Ile Leu Val His Pro Leu Phe Ser Leu Phe Ile Ile
145                 150                 155                 160

Thr Thr Ile Leu Val Asn Cys Ile Leu Met Ile Met Pro Thr Thr Pro
                165                 170                 175

Thr Val Glu Ser Thr Glu Val Ile Phe Thr Gly Ile Tyr Thr Phe Glu
            180                 185                 190

Ser Ala Val Lys Val Met Ala Arg Gly Phe Ile Leu Cys Pro Phe Thr
        195                 200                 205

Tyr Leu Arg Asp Ala Trp Asn Trp Leu Asp Phe Val Val Ile Ala Leu
    210                 215                 220

Ala Tyr Val Thr Met Gly Ile Asp Leu Gly Asn Leu Ala Ala Leu Arg
225                 230                 235                 240

Thr Phe Arg Val Leu Arg Ala Leu Lys Thr Val Ala Ile Val Pro Gly
                245                 250                 255

Leu Lys Thr Ile Val Gly Ala Val Ile Glu Ser Val Lys Asn Leu Arg
            260                 265                 270

Asp Val Ile Ile Leu Thr Met Phe Ser Leu Ser Val Phe Ala Leu Met
        275                 280                 285

Gly Leu Gln Ile Tyr Met Gly Val Leu Thr Glu Lys Cys Ile Lys Lys
    290                 295                 300

Phe Pro Leu Asp Gly Ser Trp Gly Asn Leu Thr Asp Glu Asn Trp Asp
305                 310                 315                 320

Tyr His Asn Arg Asn Ser Ser Asn Trp Tyr Ser Glu Asp Glu Gly Ile
                325                 330                 335

Ser Phe Pro Leu Cys Gly Asn Ile Ser Gly Ala Gly Gln Cys Asp Asp
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                340                 345                 350

Asp Tyr Val Cys Leu Gln Gly Phe Gly Pro Asn Pro Asn Tyr Gly Tyr
        355                 360                 365

Thr Ser Phe Asp Ser Phe Gly Trp Ala Phe Leu Ser Ala Phe Arg Leu
    370                 375                 380

Met Thr Gln Asp Phe Trp Glu Asp Leu Tyr Gln Leu Val Leu Arg Ala
385                 390                 395                 400

Ala Gly Pro Trp His Met Leu Phe Phe Ile Val Ile Ile Phe Leu Gly
                405                 410                 415

Ser Phe Tyr Leu Val Asn Leu Ile Leu Ala Ile Val Ala Met Ser Tyr
            420                 425                 430

Asp Glu Leu Gln Arg Lys Ala Glu Glu Glu Glu Ala Ala Glu Glu Glu
        435                 440                 445

Ala Ile Arg Glu Ala Glu Glu Ala Ala Ala Ala Lys Ala Ala Lys Leu
    450                 455                 460

Glu Glu Arg Ala Asn Ala Gln Ala Gln Ala Ala Ala Asp Ala Ala Ala
465                 470                 475                 480

Ala Glu Glu Ala Ala Leu His Pro Glu Met Ala Lys Ser Pro Thr Tyr
                485                 490                 495

Ser Cys Ile Ser Tyr Glu Leu Phe Val Gly Gly Glu Lys Gly Asn Asp
            500                 505                 510

Asp Asn Asn Lys Glu Lys Met Ser Ile Arg Ser Val Glu Val Glu Ser
        515                 520                 525

Glu Ser Val Ser Val Ile Gln Arg Gln Pro Ala Pro Thr Thr Ala His
    530                 535                 540

Gln Ala Thr Lys Val Arg Lys Val Ser Thr Tyr Thr Ile Arg Asn Gly
545                 550                 555                 560

Arg Gly Arg Phe Gly Ile Pro Gly Ser Asp Arg Lys Pro Leu Val Leu
                565                 570                 575

Ser Thr Tyr Gln Asp Ala Gln Gln His Leu Pro Tyr Ala Asp Asp Ser
            580                 585                 590

Asn Ala Val Thr Pro Met Ser Glu Glu Asn Gly Ala Ile Ile Val Pro
        595                 600                 605

Val Tyr Tyr Gly Asn Leu Gly Ser Arg His Ser Ser Tyr Thr Ser His
    610                 615                 620

Gln Ser Arg Ile Ser Tyr Thr Ser His Gly Asp Leu Leu Gly Gly Met
625                 630                 635                 640

Ala Val Met Gly Val Ser Thr Met Thr Lys Glu Ser Lys Leu Arg Asn
                645                 650                 655

Arg Asn Thr Arg Asn Gln Ser Val Gly Ala Thr Asn Gly Gly Thr Thr
            660                 665                 670

Cys Leu Asp Thr Asn His Lys Leu Asp His Arg Asp Tyr Glu Ile Gly
        675                 680                 685
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Leu Glu Cys Thr Asp Glu Ala Gly Lys Ile Lys His His Asp Asn Pro
    690                 695                 700

Phe Ile Glu Pro Val Gln Thr Gln Thr Val Val Asp Met Lys Asp Val
705                 710                 715                 720

Met Val Leu Asn Asp Ile Ile Glu Gln Ala Ala Gly Arg His Ser Arg
                725                 730                 735

Ala Ser Asp Arg Gly Val Ser Val Tyr Tyr Phe Pro Thr Glu Asp Asp
            740                 745                 750

Asp Glu Asp Gly Pro Thr Phe Lys Asp Lys Ala Leu Glu Val Ile Leu
        755                 760                 765

Lys Gly Ile Asp Val Phe Cys Val Trp Asp Cys Cys Trp Val Trp Leu
    770                 775                 780

Lys Phe Gln Glu Trp Val Ser Leu Ile Val Phe Asp Pro Phe Val Glu
785                 790                 795                 800

Leu Phe Ile Thr Leu Cys Ile Val Val Asn Thr Met Phe Met Ala Met
                805                 810                 815

Asp His His Asp Met Asn Lys Glu Met Glu Arg Val Leu Lys Ser Gly
            820                 825                 830

Asn Tyr Phe Phe Thr Ala Thr Phe Ala Ile Glu Ala Thr Met Lys Leu
        835                 840                 845

Met Ala Met Ser Pro Lys Tyr Tyr Phe Gln Glu Gly Trp Asn Ile Phe
    850                 855                 860

Asp Phe Ile Ile Val Ala Leu Ser Leu Leu Glu Leu Gly Leu Glu Gly
865                 870                 875                 880

Val Gln Gly Leu Ser Val Leu Arg Ser Phe Arg Leu Leu Arg Val Phe
                885                 890                 895

Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Leu Leu Ile Ser Ile Met
            900                 905                 910

Gly Arg Thr Met Gly Ala Leu Gly Asn Leu Thr Phe Val Leu Cys Ile
        915                 920                 925

Ile Ile Phe Ile Phe Ala Val Met Gly Met Gln Leu Phe Gly Lys Asn
    930                 935                 940

Tyr His Asp His Lys Asp Arg Phe Pro Asp Gly Asp Leu Pro Arg Trp
945                 950                 955                 960

Asn Phe Thr Asp Phe Met His Ser Phe Met Ile Val Phe Arg Val Leu
                965                 970                 975

Cys Gly Glu Trp Ile Glu Ser Met Trp Asp Cys Met Tyr Val Gly Asp
            980                 985                 990

Val Ser Cys Ile Pro Phe Phe Leu Ala Thr Val Val Ile Gly Asn Leu
        995                 1000                1005

Val Val Leu Asn Leu Phe Leu Ala Leu Leu Leu Ser Asn Phe Gly Ser
    1010                1015                1020

Ser Ser Leu Ser Ala Pro Thr Ala Asp Asn Asp Thr Asn Lys Ile Ala
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1025                1030                1035                1040

Glu Ala Phe Asn Arg Ile Gly Arg Phe Lys Ser Trp Val Lys Arg Asn
                1045                1050                1055

Ile Ala Asp Cys Phe Lys Leu Ile Arg Asn Lys Leu Thr Asn Gln Ile
            1060                1065                1070

Ser Asp Gln Pro Ser Glu His Gly Asp Asn Glu Leu Glu Leu Gly His
        1075                1080                1085

Asp Glu Ile Leu Ala Asp Gly Leu Ile Lys Lys Gly Ile Lys Glu Gln
    1090                1095                1100

Thr Gln Leu Glu Val Ala Ile Gly Asp Gly Met Glu Phe Thr Ile His
1105                1110                1115                1120

Gly Asp Met Lys Asn Asn Lys Pro Lys Lys Ser Lys Tyr Leu Asn Asn
                1125                1130                1135

Ala Thr Asp Asp Asp Thr Ala Ser Ile Asn Ser Tyr Gly Ser His Lys
            1140                1145                1150

Asn Arg Pro Phe Lys Asp Glu Ser His Lys Gly Ser Ala Glu Thr Met
        1155                1160                1165

Glu Gly Glu Glu Lys Arg Asp Ala Ser Lys Glu Asp Leu Gly Leu Asp
    1170                1175                1180

Glu Glu Leu Asp Glu Glu Gly Glu Cys Glu Glu Gly Pro Leu Asp Gly
1185                1190                1195                1200

Asp Ile Ile Ile His Ala His Asp Glu Asp Ile Leu Asp Glu Tyr Pro
                1205                1210                1215

Ala Asp Cys Cys Pro Asp Ser Tyr Tyr Lys Lys Phe Pro Ile Leu Ala
            1220                1225                1230

Gly Asp Asp Asp Ser Pro Phe Trp Gln Gly Trp Gly Asn Leu Arg Leu
        1235                1240                1245

Lys Thr Phe Arg Leu Ile Glu Asp Lys Tyr Phe Glu Thr Ala Val Ile
    1250                1255                1260

Thr Met Ile Leu Met Ser Ser Leu Ala Leu Ala Leu Glu Asp Val His
1265                1270                1275                1280

Leu Pro Gln Arg Pro Ile Leu Gln Asp Ile Leu Tyr Tyr Met Asp Arg
                1285                1290                1295

Ile Phe Thr Val Ile Phe Phe Leu Glu Met Leu Ile Lys Trp Leu Ala
            1300                1305                1310

Leu Gly Phe Lys Val Tyr Leu Thr Asn Ala Trp Cys Trp Leu Asp Phe
        1315                1320                1325

Val Ile Val Met Val Ser Leu Ile Asn Phe Val Ala Ser Leu Val Gly
    1330                1335                1340

Ala Gly Gly Ile Gln Ala Phe Lys Thr Met Arg Thr Leu Arg Ala Leu
1345                1350                1355                1360

Arg Pro Leu Arg Ala Met Ser Arg Met Gln Gly Met Arg Val Val Val
                1365                1370                1375
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Asn Ala Leu Val Gln Ala Ile Pro Ser Ile Phe Asn Val Leu Leu Val
            1380                1385                1390

Cys Leu Ile Phe Trp Leu Ile Phe Ala Ile Met Gly Val Gln Leu Phe
        1395                1400                1405

Ala Gly Lys Tyr Phe Lys Cys Glu Asp Met Asn Gly Thr Lys Leu Ser
    1410                1415                1420

His Glu Ile Ile Pro Asn Arg Asn Ala Cys Glu Ser Glu Asn Tyr Thr
1425                1430                1435                1440

Trp Val Asn Ser Ala Met Asn Phe Asp His Val Gly Asn Ala Tyr Leu
                1445                1450                1455

Cys Leu Phe Gln Val Ala Thr Phe Lys Gly Trp Ile Gln Ile Met Asn
            1460                1465                1470

Asp Ala Ile Asp Ser Arg Glu Val Asp Lys Gln Pro Ile Arg Glu Thr
        1475                1480                1485

Asn Ile Tyr Met Tyr Leu Tyr Phe Val Phe Phe Ile Ile Phe Gly Ser
    1490                1495                1500

Phe Phe Thr Leu Asn Leu Phe Ile Gly Val Ile Ile Asp Asn Phe Asn
1505                1510                1515                1520

Glu Gln Lys Lys Lys Ala Gly Gly Ser Leu Glu Met Phe Met Thr Glu
                1525                1530                1535

Asp Gln Lys Lys Tyr Tyr Ser Ala Met Lys Lys Met Gly Ser Lys Lys
            1540                1545                1550

Pro Leu Lys Ala Ile Pro Arg Pro Arg Trp Arg Pro Gln Ala Ile Val
        1555                1560                1565

Phe Glu Ile Val Thr Asp Lys Lys Phe Asp Ile Ile Ile Met Leu Phe
    1570                1575                1580

Ile Gly Leu Asn Met Phe Thr Met Thr Leu Asp Arg Tyr Asp Ala Ser
1585                1590                1595                1600

Asp Thr Tyr Asn Ala Val Leu Asp Tyr Leu Asn Ala Ile Phe Val Val
                1605                1610                1615

Ile Phe Ser Ser Glu Cys Leu Leu Lys Ile Phe Ala Leu Arg Tyr His
            1620                1625                1630

Tyr Phe Ile Glu Pro Trp Asn Leu Phe Asp Val Val Val Val Ile Leu
        1635                1640                1645

Ser Ile Leu Gly Leu Val Leu Ser Asp Ile Ile Glu Lys Tyr Phe Val
    1650                1655                1660

Ser Pro Thr Leu Leu Arg Val Val Arg Val Ala Lys Val Gly Arg Val
1665                1670                1675                1680

Leu Arg Leu Val Lys Gly Ala Lys Gly Ile Arg Thr Leu Leu Phe Ala
                1685                1690                1695

Leu Ala Met Ser Leu Pro Ala Leu Phe Asn Ile Cys Leu Leu Leu Phe
            1700                1705                1710

Leu Val Met Phe Ile Phe Ala Ile Phe Gly Met Ser Phe Phe Met His
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1715 1720 1725

Val Lys Glu Lys Ser Gly Ile Asn Asp Val Tyr Asn Phe Lys Thr Phe
1730 1735 1740

Gly Gln Ser Met Ile Leu Leu Phe Gln Met Ser Thr Ser Ala Gly Trp
1745 1750 1755 1760

Asp Gly Val Leu Asp Ala Ile Ile Asn Glu Glu Ala Cys Asp Pro Pro
1765 1770 1775

Asp Asn Asp Lys Gly Tyr Pro Gly Asn Cys Gly Ser Ala Thr Val Gly
1780 1785 1790

Ile Thr Phe Leu Leu Ser Tyr Leu Val Ile Ser Phe Leu Ile Val Ile
1795 1800 1805

Asn Met Tyr Ile Ala Val Ile Leu Glu Asn Gly Ile
1810 1815 1820

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 521 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGAGCCGCA TGCAGGGCAT GAGGGTACGT ACCACCCTGT GCTGCCGACA ACACCCTATC 60

GCTCATCCAT CCACCACACA CTTCGCTCCA CACTTCACAT TCACATTTCT ATTTCAACTT 120

CTACGATCAT TTTTTAACAT TTTAAAATTT CCAACGTRCC AGCCGTACTM GGGCTCCTTT 180

TTTCGATATT TCTGCATSAA TCACCGGATC AAAATTTGTT TTTAATAGTT AATTTGGACA 240

GTTATCCGAT TCATTGGCAG TAGTCGATTG AAGTAATTAT TAGTGAATCA TTTTGAAGTG 300

GTCGGTGGCA CCCCTGAATG GCTTAGTATC ATCACTGTTC GTCATAAACC TCTTTTAGAA 360

AGGGTCAATG GGATTTATTG TGGAGAGATA TTYRTCCATG TTTTGGTCTC TTTTCTATTG 420

GTCTTATTAT TAGCTAGATT AGACTTTTGT AATTACTTAG TTATTTGGAA TGCTAATTTA 480

TATTCTGCAC CTTAGATTTT TTCTTCTTGT ATCTTCATCG A 521

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 568 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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GCTAACTGCT ACATAGTTAC TGCACAGTAT TAATGACATT AACGTCCTTA TATCCCAACT      60
AATAATGCGC CCACTAACAA ATGCACGCCA TTGATATAAG AAAGGAGACG TATCAGTACT     120
TCCAATATAT CCTTCGTGAC CAGTGTAGTA ATACGTACGT ATGTGACAGG TGGTGGTAAA     180
CGCTCTCGTG CAAGCGATCC CGTCCATCTT CAACGTGTTG TTGGTGTGTC TTATCTTCTG     240
GCTGATCTTC GCCATCATGG GAGTACAACT GTTCGCTGGC AAATATTTCA AGGTATTAAT     300
TTATTAACAT AACAAAAAAA TATTTCAATT CGTAAAATCT TATTAGTGTG TTCAAAATTT     360
CTAACATGTT TTTCTTTGTT CTGTTCTAGT GCGTCGACCT CAACCACACG ACGTTGAGCC     420
ACGAAATCAT CCCAGACCGG AATGCGTGCA TCTTAGAGAA CTACACCTGG GAGAACTCAC     480
CGATGAACTT TGACCATGTC GGCAAGGCGT ATCTCTGCCT GTTCCAAGTG GCCACCTTCA     540
AGGGATGGAT ACAGATCATG AACGACGC                                        568
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Claims

1. An isolated nucleic acid fragment comprising a nucleic acid sequence encoding a non-dipteran sodium channel, or portion thereof.

2. The fragment of Claim 1 in which the channel is either lepidopteran, coleopteran or homopteran.

3. The fragment of Claim 2 which is lepidopteran.

4. The fragment of Claim 3 which is derived from Heliothis, Helicoverpa or Spodoptera.

5. The fragment of Claim 4 which is derived from Heliothis virescens, Heliothis armigera, or Helicoverpa zea.

6. The fragment of Claim 1 which hybridizes with a nucleic acid sequence depicted in Figure 1 under medium or high stringency conditions.

7. The fragment of Claim 1 which comprises all or a portion of the sequence depicted in Figure 1.

8. The fragment of Claim 1 which is capable of being used as a probe to detect RFLPs in an insect population comprising both pyrethroid sensitive and pyrethroid resistant individuals.

9. The fragment of Claim 1 which is detectably labelled.

10. An isolated nucleic acid fragment deposited with the American Type Culture Collection under Accession No. 75334.

11. A vector comprising the fragment of Claim 1.

12. A host cell comprising the vector of Claim 11.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number EP 93118061.6

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	CHEMICAL ABSTRACTS, vol. 116, no. 3, January 20, 1992, Columbus, Ohio, USA DOYLE D.E. et al. "PCR-based phylogenetic walking: isolation of para-homologous sodium channel gene sequences from seven insect species and an arachnid" page 129 abstract-no. 16 363v & Insect. Biochem. 1991, 21(6), 689-96 ----	1,8	C 07 H 21/00 C 12 Q 1/68
			TECHNICAL FIELDS SEARCHED (Int. Cl.5) C 07 H C 12 Q

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	31-03-1994	SCHNASS

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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